A STUDY ON THE EFFICACY AND CORNEAL PENETRATION OF BESIFLOXACIN IN ROUTINE CATARACT SURGERY CASES

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in

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Submitted by

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PERIYAR COLLEGE OF PHARMACEUTICAL SCIENCES

TIRUCHIRAPPALLI – 620 021.

An ISO 9001:2015 Certified Institution

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CERTIFICATE

This is to certify that the thesis entitled **"A STUDY ON THE EFFICACY AND CORNEAL PENETRATION OF BESIFLOXACIN IN ROUTINE CATARACT SURGERY CASES"** submitted by **B. NIVETHA, *B. Pharm.,*** during September 2018 for the award of the degree of **"MASTER OF PHARMACY *in* PHARMACY PRACTICE"** under the **Tamilnadu Dr.M.G.R. Medical University**, Chennai is a *bonafide* record of research work done in the Department of Pharmacy Practice, Periyar College of Pharmaceutical Sciences and at Vasan Eye Care Hospital, Tiruchirappalli under my guidance and direct supervision during the academic year 2017-18**.**

Place: Tiruchirappalli – 21.

Date: 10th Sep 2018 **(Dr. A. M. ISMAIL)**

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Place: Tiruchirappalli – 21.

Date**:** 10th Sep 2018 **(Dr. R. SENTHAMARAI)**

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ABBREVIATIONS USED

ABBREVIATION	EXPANSION
AC	Anterior Chamber
ADR	Adverse Drug Reaction
AIIMS	All India Institute of Medical Sciences
BAK	Benzalkonium chloride
CCC	Continuous curved capsulorrhexis
CDC	Crater Divide and Conquer
CI	Confidence interval
Cm	Chloramphenicol
CME	Cystoid macular edema
CSC	Cataract surgical coverage
CSR	Cataract surgical rate
ESCRS	European Society of Cataract and Refractory Surgeons
FDA	Food and Drug Administration
ICB	Iris and Ciliary Body
IEC	Institutional Ethics Committee
IOL	Intraocular Lens
IOP	Intraocular Pressure
LA	Local Anaesthetic
LD50	Lethal Dose 50
MAC	MacConkey Agar
MIC	Minimum Inhibitory Concentration
MRSA	Methicillin-resistant *Staphylococcus aureus*

MRSE	Methicillin-resistant *Staphylococcus epidermidis*
NOAEL	No Observed Adverse Effect Level
PAGE	Polyacrylamide gel electrophoresis
PSA	Polar surface area
R/L	Right / Left
RAAB method	Rapid Assessment of Avoidable Blindness method
RPEC	Rajendra Prasad Eye Centre
SDS	Sodium Dodecyl Sulphate
SRR	Sight Restoration Rate (per year)
TASS	Toxic anterior shock syndrome
TDC	Trench, Divide and Conquer
Vd	Apparent volume of distribution
WHO	World Health Organization

Introduction

I. INTRODUCTION

CATARACT

Cataract is an eye disease in which the clear lens of the eye becomes cloudy or opaque, causing a decrease in vision. Although the word *cataract* to describe this condition has been part of the English language since only the 15th century, the eye disease has been recognized and surgically treated since ancient times.

According to WHO: "Cataract is clouding of the lens of the eye which impedes the passage of light. Although most cases of cataract are related to the ageing process, occasionally children can be born with the condition, or a cataract may develop after eye injuries, inflammation, and some other eye diseases."The lens is a portion of the eye that is normally clear. It focuses rays of light entering the eye onto the retina, the light-sensitive tissue at the back of the eye. In order to get a clear image onto the retina, the portions of the eye in front of the retina, including the lens, must be clear and transparent. The light striking the retina initiates a chemical reaction within the retina. The chemical reaction, in turn, initiates an electrical response which is carried to the brain through optic nerve. The brain then interprets what the eye sees.

In a normal eye, light passes through the transparent lens to the retina. The lens must be clear for the retina to receive a sharp image. If the lens is cloudy from a cataract, the image striking the retina will be blurry or distorted and the vision will be blurry. The extent of the visual disturbance is dependent upon the degree of cloudiness of the lens.

Most cataracts are related to ageing. Cataracts are very common in older people. By age 80, more than half of all Americans either have some degree of cataract or have already undergone cataract surgery in one or both eyes. By age 95, this percentage increases to almost 100%. A cataract can occur in either or both eyes. Individuals with a cataract in one eye usually go on to develop a cataract in the other eye as well. A cataract is not contagious and cannot spread from one eye to the other or from person to person. Cataracts do not cause the eye to tear abnormally. They are neither painful nor make the eye itchy or red.

Although vision can be restored in most people with cataracts, age-related cataracts are still the most common cause of blindness in the world, primarily because many third-world nations lack appropriate and available surgical services.

Fig.1

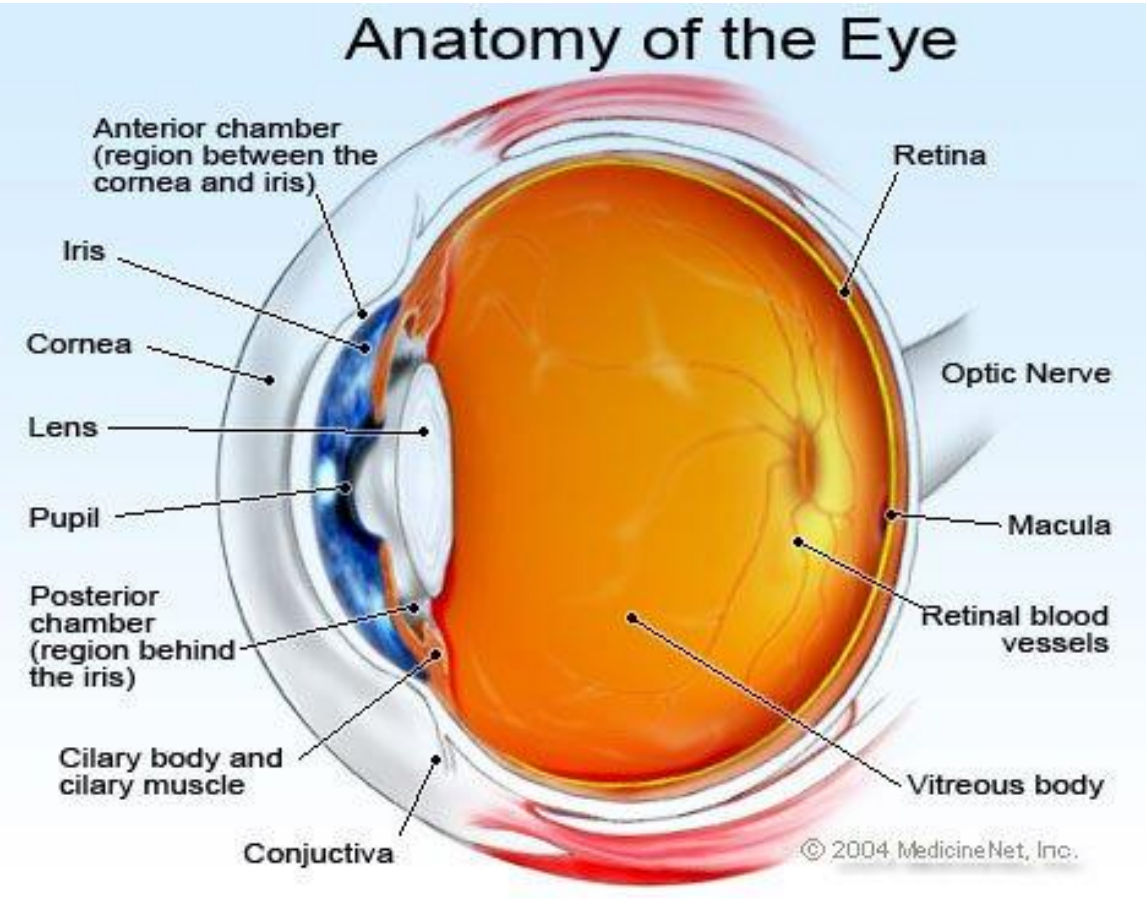
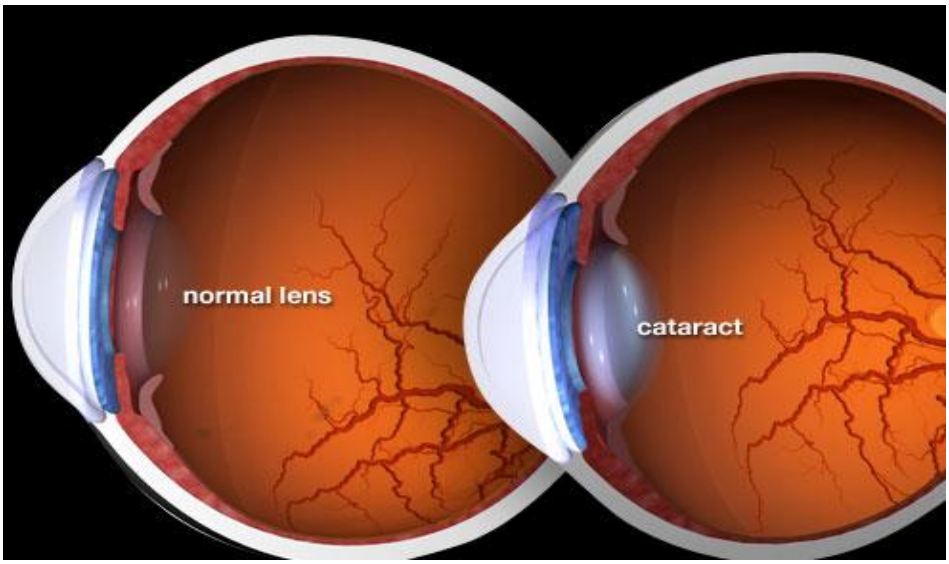


Fig. 2

DIFFERENCE BETWEEN NORMAL VISION AND CATARACT VISION

Fig.3



LENS – NORMAL & CATARACT

TYPES OF CATARACT

- **Nuclear cataracts**, which form in the lens' nucleus, are the most common type of cataracts. Because opacity develops in the centre of the lens, known as the central nucleus, nuclear cataracts interfere with a person's ability to see objects in the distance. Usually the result of advancing age, nuclear cataracts can take years to develop and often give the nucleus a yellow tint.

- **Cortical cataracts** begin at the outer rim of the lens and gradually work toward the central core. Thus, this type of cataract resembles spokes of a wheel. Patients with cortical cataracts often notice problems with glare, or a "halo" effect around lights. They may also experience a disruption of both near and distance vision.

- **Subcapsular cataracts** progress the most rapidly. While nuclear cataracts take years to develop, subcapsular cataracts reach an advanced stage within a matter of months. Posterior subcapsular cataracts affect the back of the lens, causing glare and blurriness. This type of cataract is usually seen in patients who suffer from diabetes, extreme nearsightedness or retinitis pigmentosa, as well as among those who take steroid medication.

- **Congenital cataracts** refer to cataracts that are present from birth, as well as to those that develop in early childhood. These cataracts can be nuclear, cortical, or subcapsular. Congenital cataracts may be linked to an infection contracted by the mother during pregnancy or to a genetic condition such as Fabry disease, Alport syndrome, or galactosemia. Because clear vision is essential to the development of the child's eyes and brain, it is important to diagnose congenital cataracts as early as possible.

- **Secondary cataracts** are caused by disease or medications. Diseases that are linked with the development of cataracts include glaucoma and diabetes. The use of the steroid prednisone and other medications can sometimes lead to cataracts.

- **Traumatic cataracts** develop after an injury to the eye, but it can take several years for this to happen.

- **Radiation cataracts** can form after a person undergoes radiation treatment for cancer.
  Because each of these cataract types affects vision in a distinct manner, it is possible for a patient to have more than one type of cataract at the same time.

STAGES OF CATARACT

Early Stages of Cataracts

When cataracts first develop, they are typically small enough that they don't impair vision. However, if left untreated, cataracts can lead to bigger vision problems. To help you preserve your eyesight, it's best to catch and treat this issue in its early stages.

The first signs of cataracts typically include slightly blurred vision, faded colors, minor loss of night vision, and halos around lights. Other symptoms in the early stages of cataracts include an increase of glare and double vision.

On top of that, a person with cataracts may have a more difficult time adjusting when going from a brightly lit room to a darker one. This is because a cataract can cause decreased contrast sensitivity, or the eye's ability to adjust to different light levels intensities.

When a cataract forms, it will also continue to grow and affect your vision. If your eyesight seems to be getting worse to a point where you need stronger prescriptions for eye glasses and contact lenses, contact your doctor as this could be an early sign of cataracts.

Advanced Stages of Cataracts

As cataracts progress, symptoms will increase and become more severe. In the advanced stages of cataracts, it becomes more difficult to see clearly. Your vision will become cloudier, especially during the day. You may also see a visible white spot on the lens of the eye and milky or yellowish pupils.

In some rare cases, an advanced cataract can start to leak into other parts of the eye. This can lead to inflammation, pain, and most significantly, complete loss of eyesight.

Signs and symptoms of cataracts include:

- Clouded, blurred, foggy or dim vision
- Increasing difficulty with vision at night
- Sensitivity to light and glare
- Need for brighter light for reading and other activities
- Seeing "halos" around lights
- Frequent changes in eyeglass or contact lens prescription
- Fading or yellowing of colors
- Double vision in a single eye
- Near sightedness (in older people)
- Problems with glare during the day
- Double vision in the affected eye

Risk factors

Factors that increase your risk of cataracts include:

- Increasing age
- Diabetes
- Excessive exposure to sunlight
- Smoking
- Obesity
- High blood pressure
- Previous eye injury or inflammation
- Previous eye surgery
- Prolonged use of corticosteroid medications
- Drinking excessive amounts of alcohol
- Statin medicines used to reduce cholesterol
- Hormone replacement therapy
- Significant alcohol consumption
- High Myopia
- Family history

Causes

Most cataracts develop when ageing or injury changes the tissue that makes up the eye's lens. Some inherited genetic disorders that cause other health problems can increase your risk of cataracts. Cataracts can also be caused by other eye conditions, past eye surgery or medical conditions such as diabetes. Long-term use of steroid medications, too, can cause cataracts to develop.

Pathophysiology

Changes in the lens proteins (crystallins) affect how the lens refracts light and reduce its clarity, therefore decreasing visual acuity. Chemical modification of these lens proteins leads to the change in lens colour. New cortical fibres are produced concentrically and lead to thickening and hardening of the lens in nuclear sclerosis, which often appears yellow and can increase the focusing power of the natural lens. Increasing myopia can also be evidence of a

progressing nuclear sclerotic cataract. In an experimental model, oxidative stress contributed to cataract formation, causing a decrease in the level of adenosine triphosphate and glutathione disulfide.

Cortical cataracts are most often seen as whitish spokes peripherally in the lens, separated by fluid. Vacuoles and water clefts can also be seen in these lenses. Posterior subcapsular cataracts are due to the migration and enlargement of lens epithelial cells (Wedl cells) posteriorly. Diabetes mellitus is a major factor in the formation of this type of cataract. Osmotic stress due to sorbitol accumulation has been linked with sudden worsening in patients with uncontrolled hyperglycemia. However, research has also found that when sorbitol dehydrogenase was blocked, preventing sorbitol accumulation, oxidative stress was connected with slow-developing cataracts.

Aetiology

Although the most common cause of cataract is the normal ageing process, other conditions that can contribute to opacification of the lens include trauma, metabolic disorders (hereditary or acquired), infections (e.g., *rubella*), medications, or congenital problems. While there is still some debate, smoking, alcohol, and exposure to UV radiation have also been indicated as factors that may cause cataract progression, especially nuclear sclerotic cataracts.

Etiopathogenesis

Clinically, cataract patients can be classified into morphological groups *viz*. nuclear, sub-capsular or cortical for studying the risk factors. Most case-control studies have been on pooled cataracts. This approach has been strongly criticized by some authors on the basis that different morphological types have different risk factors. Since cataract is a major cause of avoidable blindness in the developing countries, the key to the success of the Global Vision 2020: The right to sight initiative is a special effort to tackle cataract blindness by finding out precise cause. Even though effective surgical procedures are available for treatment, the problem of post-operative complications, cost of surgery, and high number of people requiring surgery pose a substantial economic burden.

It has been estimated that delaying cataract onset by 10 years could reduce the need for surgery by as much as half. The respective causes of different type of cataracts must be known in order to understand the patho-physiology of disease and its management. However,

risk factors for cataracts summarized above cannot be comprehended this way, but they may help to confer the matter with new approaches.

Inherited disorders are often involved in the development of congenital cataracts in children with ratio of 1:10,000 births. Such cataracts are most often due to inborn abnormalities in the structure or shape of the lens capsule. *PITX3* gene has been reported as responsible for some inherited cataracts. The role of Osaka variant is new interest of point at present. Infantile cataracts, those developing within the first year of life, are frequently associated with a metabolic or systemic disease. The role of gender and race or community could not be made clear in the development of cataract. Age-related cataracts are mostly developed due to increase in oxidative stress in lens due to various systemic diseases or imbalance in pro and anti-oxidants in body particularly eyes.

Trauma has direct impact to induce denaturation processes in eye lenses. Removal and implant placement can be complicated in these cases though, as the blunt force often tears the zonular support. Complications of untreated systemic as well as local conditions are well-elaborated in development of cataract, though their mechanism is still unclear.

Cataracts, those precede by metabolic abnormalities, are mostly associated with congenital aberrations. Most of them are having peculiar diagnostic characters like oil droplet type of opacity appearance in galactosemia, multicoloured crystals-like opacities can be seen in hypocalcemia and sunflower-like appearance in copper metabolic error. The deficiencies of micronutrients directly affect the antioxidant systems in eyes lens. The role of nutrition in cataract formation in developing countries is perhaps closely linked with diarrhoea and poverty, all of which are closely interrelated.

Many drug abuses as well as various toxins may cause oxidative damage and interrupt the lens growth. They bind to sulfhydryl groups, including glutathione peroxidase and N^{a+} K^{+} ATPase, along with super oxide dismutase and catalase, which are responsible for the maintenance of clarity of the lens during oxidative stress. Most of them are reversible in nature.

Radiation stimulates the senile changes in eye lenses. Radiation or electromagnetic waves can rouse the exfoliation process in lens that leads to disturbance in protein arrangement and oxidative systems. Experimental evidence indicates maximum lens sensitivity to UVR-B in the wavelength region around 300 nm. ROS are mediators of damage induced by UVR and

can trigger alteration in growth factors- and cytokine-mediated signal transduction pathways, leading to aberrant gene expression.

Elimination of causes of cataract which were described above may reverse the cataractous changes in the initial stage. Nutritional supplements and balancing antioxidants during old age and malnutrition and in condition of diarrhoea are reported in preventing senile cataract. Correction of transient metabolic defects e.g., treatment of galactosemia, copper metabolism etc., are also found useful in the prevention of cataract. Eluding factors affecting congenital defects like consanguineous marriage, galactosemic diet, medication and radiation during pregnancy can be helpful in preventing development of congenital cataract.

Prescription of alternate medications for steroid and other drugs prone to cataract formation can be used to prevent cataractous changes. Many studies reported that antioxidants (Vit E, Vitamin- C, thiamine, riboflavin, lutein, flavonoids, carotenoids etc.) can effectively prevent and cure UVB-induced protein oxidation and photo-peroxidation of lipids in lens. Local protective measures like use of UV-protected sun glasses as well as use of UV-absorbing hydrogel polymers also can be useful in this way. Though many of the factors identified are responsible for the development of cataract, but their mechanism of action is still unclear.

How a cataract forms

The lens, where cataracts form, is positioned behind the colored part of your eye (iris). The lens focuses light that passes into your eye, producing clear, sharp images on the retina. As one ages, the lenses become less flexible, less transparent and thicker. Age-related and other medical conditions cause tissues within the lens to break down and clump together, clouding small areas within the lens.

As the cataract continues to develop, the clouding becomes denser and involves a bigger part of the lens. A cataract scatters and blocks the light as it passes through the lens, preventing a sharply defined image from reaching your retina. As a result, the vision becomes blurred.

Cataracts generally develop in both eyes, but not evenly. The cataract in one eye may be more advanced than the other, causing a difference in vision between eyes.

Diagnosis

Cataracts are fairly easy to diagnose. Nevertheless, for the most accurate diagnosis, there are a number of advanced tests that your doctor may use. Utilizing these diagnostic tools, your doctor can also check for an additional eye disease, such as glaucoma or macular degeneration.

Visual Acuity Test

The **visual acuity test** is used to determine the smallest letters one can read on a standardized **chart** (Snellen **chart**) or a card held 20 feet (6 meters) away. Special charts are used when testing at distances shorter than 20 feet (6 meters). Some Snellen charts are actually video monitors showing letters or images. This test allows detection of any significant changes in the vision.

If one is already been diagnosed with cataracts, and the vision has degenerated to 20/40 or below, doctor may recommend surgery. In some cases, one may have a good score on this test, but may still present with other cataract symptoms, such as light sensitivity or blurred vision. When this occurs, ophthalmologist will likely recommend more tests to determine whether cataracts are, in fact, responsible for those symptoms.

Slit Lamp Exam

Surgeon will give eye drops to dilate the pupil. By shining a light on the front of the eye, we can usually detect any white spots on the lens, even if they are very minute. The slit lamp exam often allows for extremely early diagnosis, so that an ophthalmologist can detect cataracts before they begin to affect the vision.

During the test, the surgeon will likely use a magnifying lens to examine the retina and optic nerve. Although this step is not necessary for cataract detection, it enables surgeon to check for other serious eye conditions.

Retinal Exam

To prepare for a retinal exam, eye surgeon puts drops in the eyes to dilate the pupils wide. This makes it easier to examine the retina. Using a slit lamp or an ophthalmoscope, eye surgeon can examine the lens for signs of a cataract.

Glare and Contrast Sensitivity Tests

These two tests are similar to a traditional visual acuity test.

During a **Glare test**, they will be asked to read the Snellen chart under various lighting conditions. If we have difficulty discerning the letters under very bright light, this could be a good indication of cataracts. The **Contrast sensitivity tests** use different kinds of charts; instead of the letters getting smaller as they go down the chart, they "fade," or contrast less with the white background. If we are unable to read all but the most defined rows of letters, this, too, could be an indication of cataracts.

Ishihara Color Test

Named after its designer, Dr.Shinobu Ishihara, this test uses a series of plates covered with colored dots. The dots vary in color, forming a number in the centre of the plate. Those with an inability to distinguish hues on the blue-green end of the spectrum will not be able to see the numbers. If have not been previously diagnosed as color blind, and we do not pass the Ishihara color test, may be suffering from advanced cataracts.

Tonometry Test

Ophthalmologist will direct a puff of air towards the eye, and it will measure the intraocular pressure (IOP), determined by the amount of vitreous fluid inside the eye. Although tonometry tests are most commonly used to diagnose glaucoma, it is important that eye surgeon be as thorough as possible during cataract diagnosis. When left undiagnosed and untreated, glaucoma, macular degeneration, and similar eye diseases can have dramatic consequences for our vision and ocular health.

Treatment for Cataracts

The prescription glasses can't clear the vision, the only effective treatment for cataracts is surgery. In the beginning stages of cataracts, vision may be slightly improved using forms of visual correction. However, in the later stages, surgery may be required. Fortunately, surgery has proven to be extremely successful in the removal of cataracts. During cataract surgery, physician will replace the natural lens with an IOL.

Patients' responses to the presence of a cataract vary. A cataract in only one eye may be disturbing to a particular patient and may not cause significant symptoms in another patient. Cataracts usually do not harm our eye, so we can have surgery when it is convenient for us and when the cataract interferes with our daily activities. Once we understand the benefits and risks of surgery, we can make an informed decision about whether cataract surgery is right for us. In most cases, delaying cataract surgery will not cause long-term damage to the eye or make the surgery more difficult.

If the eye has other diseases that have caused visual loss such as glaucoma, macular degeneration, diabetic retinopathy, or optic nerve damage from glaucoma or other diseases, cataract surgery may not improve the vision.

If both eyes have cataracts and surgery is agreed upon, the surgery on the second eye is generally planned at least a week after the first eye. There is usually no harm in waiting a much longer period of time between the two eye surgeries. Because the lens of the eye is necessary to accurately focus light onto the retinal surface and removal of the cataract involves removal of the lens, modern cataract surgery combines removal of the lens and placement of a new artificial lens into the eye. Measurements for the size, shape, and power of this lens will be taken prior to the surgery so that the specific lens can be available for implantation at the time of surgery.

It's up to the surgeon to decide when cataract surgery is right for the patient. For most people, there is no rush to remove cataracts because they usually don't harm the eye. But cataracts can worsen faster in people with diabetes. Delaying the procedure generally won't affect how well the vision recovers if we later decide to have cataract surgery. Therefore it is advised to take time to consider the benefits and risks of cataract surgery with the surgeon.

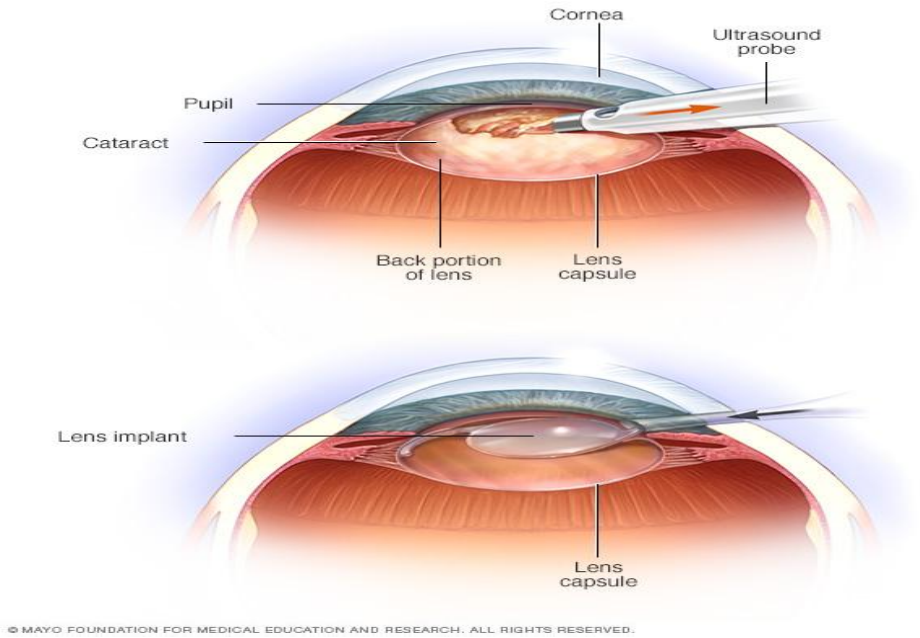
Cataract surgery

Cataract surgery involves removing the clouded lens and replacing it with a clear artificial lens. The artificial lens, called an intraocular lens (IOL), is positioned in the same place as the natural lens. It remains a permanent part of the eye. For some people, other eye problems prohibit the use of an artificial lens. In these situations, once the cataract is removed, vision may be corrected with eyeglasses or contact lenses.

Cataract surgery is generally done on an outpatient basis, a day-care procedure. During cataract surgery, eye surgeon uses a local anesthetic to numb the area around the eye, and the patient usually stays awake during the procedure. Cataract surgery is generally safe, but it carries a risk of infection and bleeding. It also increases the risk of retinal detachment. After the procedure, the patient will have some discomfort for a few days. Healing generally occurs within eight weeks. If cataract surgery is needed in both eyes, the surgeon will schedule surgery to remove the cataract in the second eye after having healed from the first surgery.

The purpose of the lens is to refract light rays that come into the eye to help us see. Having a cataract can be like looking through a foggy or dusty car windshield. Things may look blurry, hazy or less colourful.

Fig. 4



CATARACT SURGERY

PHACOEMULSIFICATION

Phacoemulsification, introduced by Kelman in 1967, is undoubtedly one of the most important innovations in ophthalmology. This has now been accepted as gold standard surgical procedure for management of cataract. this allows the removal of cataract through a 3.0 mm incision, thus eliminating many of the complications of wound healing related to large incision surgery and greatly shortens the recovery period.

Preoperative preparation

All the basics of ECCE like full mydriasis, good anaesthesia, antimicrobial preparation of operative part, use of disposable plastic drape are to be given due importance.

Patient Selection

The current technique and equipment have developed to such an extent that this technique may be used in majority of cases of cataract. However preoperative evaluation of patients under full mydriasis with slit-lamp examination is very important. This will give valuable information about pupillary dilation, grade of nuclear sclerosis and zonular support. Sometimes evaluation of fundus is possible, and will assure you a good post operative vision in case of uneventful surgery.

Relative contraindications are miotic pupil which refuses to dilate, very hard nucleus and lack of zonular support. For a beginner, ideal case is patient of around 60 years with moderate hardness and good fundal reflex.

Following are difficult situation for phacoemulsification:

- Deep set eyes
- Shallow eyes
- Hazy cornea
- Non dilating pupil
- Brunescent cataract Grade IV/V or very soft cataract
- Subluxated/ dislocated lens
- Cataract in vitrectomized patient

Hardness of Nucleus

Utmost importance should be given to assessment of the hardness of the nucleus. This can be assessed with slit-lamp examination under mydriasis. Nucleus of crystalline lens changes from transparent to gray to gray-yellow amber, brown and finally to almost black. To a certain degree, variation in colour corresponds to an increase in hardness of nucleus. Other important consideration is patient age. Older the patient harder the nucleus is likely to be.

Hardness of nucleus can be classified as follows:

1. Soft nucleus (Grade 1) - transparent to pale gray
2. Slightly hard nucleus (Grade 2) gray - greyish yellow
3. Moderately hard nucleus (Grade 3) - yellow with tinge of gray
4. Hard Nucleus (Grade 4) - Yellow amber
5. Very hard Nucleus (Grade 5) - brown-black

PHACOMACHINE

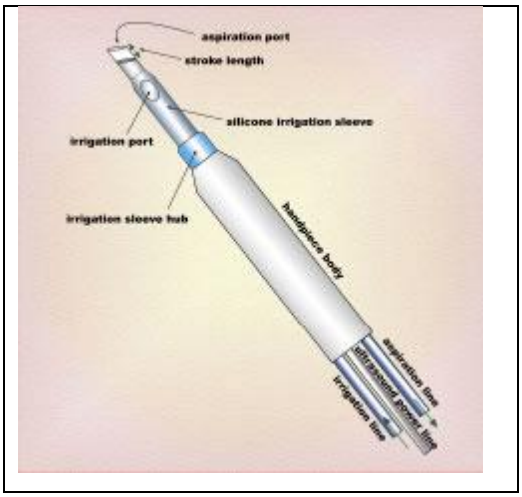
Ultrasonic handpiece: Ultrasonic power is most often produced by enclosed piezoelectric crystals which convert electricity into mechanical vibration. This energy is transmitted along the handpiece into phaco-needle in such a way that primary oscillation is axial. Irrigating fluid flows through two ports located 180° apart on the silicon sleeve surrounding the phaco tip.

Fluid flows between needle and silicon sleeve. Normal frequency of various phaco machines ranges from 20,000 to 80,000 (20-80 KHz) Hertz. Frequency is fixed for particular machine. Usually it is in the range of 40 KHz. Stroke length is the forward and backward movement of the needle along the longitudinal axis. These oscillations are between 70-120 microns wide. This can be varied by selecting different power limits.

Phaco tips - These are made up to titanium. It can have an opening angulation of 0°, 15°, 30°, and 45°. Greater angulation facilitates sculpting whereas lower angle is good for occlusion. 30° tip is suitable for both functions and is the most preferred one. External

diameter of needle is 1.1 mm and internal diameter is 0.9mm. In case of microtip needle external diameter is 0.9 mm and internal diameter is 0.5-0.7 mm. The tip is covered with silicon sleeve that insulates and protects the tissue at the incision site.

Fig. 5

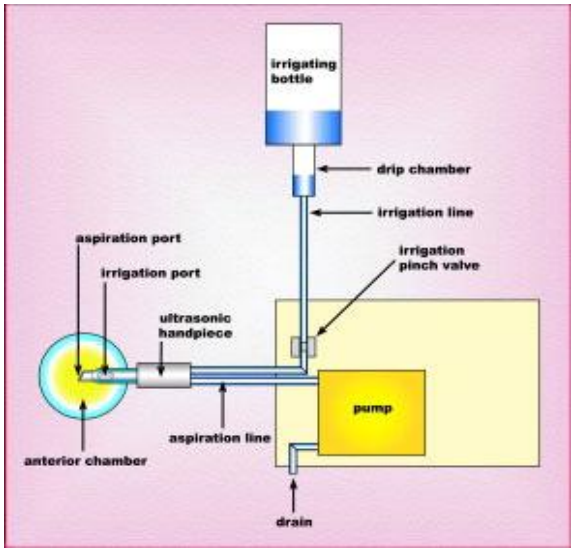


PHACO TIP

FLUID DYNAMICS

Aspiration flow rate: The quantity of liquid (measured in cubic centimeters = CC) which circulates in unit time (one minute) is defined as flow. The liquid flows downward sunder gravity from the infusion bottle, passes through infusion line and reaches the eye through handpiece. It exits the eye through the aspiration line and also through main and side port incision (leakage). The flow of fluid is mainly responsible for bringing the nucleus fragments towards the phaco tip.

Fig.6FLUID DYNAMICS



PUMPS

Pumps create the vacuum during the aspiration flow. There are basically three different types of pumps used in phaco machines.

1. Peristaltic pumps

Peristaltic pumps have been used the longest. A peristaltic pump creates aspiration by advancing fluid through a tube that is wound tightly around a 'humped' rotating wheel. It works by squeezing off small segments of the tubing between successive rollers mounted on a wheel. As the wheel turns the segment of fluid trapped between two rollers is moved, creating a vacuum behind that is relieved by more fluid coming up the tubing.

With a peristaltic pump, flow rate depends on pump speed when the tip is not occluded. Aspiration vacuum builds when the tip is partially or totally occluded, andonce again the rate of rise of the vacuum depends on the pump speed. The vacuum level limit and the flow rate (vacuum rate of rise) can be adjusted independently with peristaltic pumps. The vacuum level can be adjusted by setting the transducer to allow for venting at a certain limit. The flow rate is determined by setting a limit to the rotation speed of the peristaltic pumps. Also, if the surgeon desires, both the vacuum limit and the flow rate can be varied by linear control, which is the instantaneous change of each parameter by depression of the foot pedal.

2. Vacuum Pumps

The evacuation of air from a closed container by a vacuum pump can be used to form a vacuum reservoir. This vacuum reservoir can create a flow in the aspiration tubing. The two styles of vacuum pumps are ‘diaphragmatic' or 'venturi'.

Venturi pump

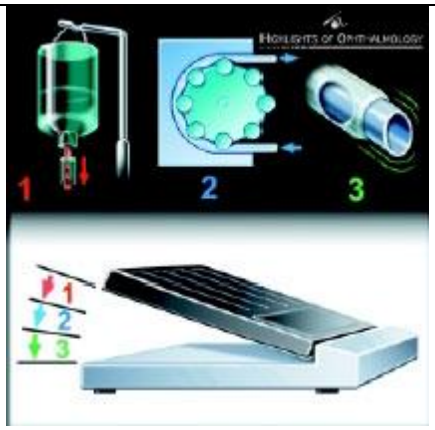
This is driven the compressed gas (nitrogen or air) that is directed through the chamber. By varying the size of the opening, the volume of the gas through the chamber is proportionately controlled. The gas flow over the opening of tube into chamber creates a pressure differential via a venturi effect with air flow. This air flow and pressure differential in the tube creates a vacuum in the chamber that pulls fluid in from the aspiration tubing. Vacuum and flow rate are proportionately linked on a venturi pump and cannot be independently adjusted as they can on a peristaltic pump.

FOOT SWITCH

Phaco machines come with a foot switch to control functions of the machine. Newer machines can almost be totally controlled with the foot switch. The basic control positions are the same with all machines. In the ultrasound mode (U/S), on depressing the foot pedal, the position entered first is 'position 1'(Fig. 7) . In this position, the irrigation line is opened, with free flow out of the phaco tip sleeve. On further depressing the foot pedal, aspiration is initiated and vacuum is transmitted to the phaco tip. This is 'position 2'. Further depression of the foot pedal activates the ultrasound oscillation of the phaco tip, generating the force required to emulsify the nucleus. The foot pedal control is linear in the sense that the power generated is proportional to the amount by which the foot pedal is depressed.

Fig. 7

FOOT SWITCH POSITIONS



Foot switch positions
1 - Irrigation on; 2 - Vacuum on;
3 - U/S Power on

Surge and venting

Surge is a phenomenon encountered in position 2 or 3 when an occluded fragment at the phaco tip is aspirated and suddenly occlusion is broken. Because of higher pressure in anterior chamber (AC) fluid suddenly rushes to lower pressure in phaco tip, creating a potential for AC collapse which could damage the cornea or posterior capsule. Newer machines have effective anti surge designs. Venting is the main anti collapse system. In this case the negative pressure inside the aspiration line is neutralized with emission of liquid or air.

INCISION CONSTRUCTION

Ideal incision for phacoemulsification should be astigmatically neutral and free from sutures.

There are following different parameter in relation to incision:

1. Position (a) with regard to limbus(b) with regard to o'clock position
2. Size
3. Shape

Position with regard to the limbus, three sites can be chosen for incision.

1. Sclero-corneal
2. Limbal
3. Clear corneal

Sclero corneal

As one moves away from centre of the cornea, surgically induced astigmatism is minimized. A large incision (5.5 mm) is also possible at this site. However, there are a number of disadvantages associated with this type of incision.

1. Conjunctiva must be reflected. Diathermy is a must
2. Greater time is required
3. Difficulty is manipulating of instrument in A.C.

Incision is constructed about 2mm away from limbus after reflecting the conjunctiva and applying diathermy. With blade or Bard Parker knife, a vertical partial thickness sclera incision is given. With crescent knife, dissection is made in sclera and cornea and finally is entered with a 3 mm keratome.

Limbal incision

Limbal incision is one where external edge is localized 0.5mm posterior of vascular arcade that is on fixed conjunctiva. These incisions are also called as 'near clear incision'.

Advantages of limbal incision are:

a) They induce less astigmatism as away from visual axis
b) They heal rapidly as they have a vascular support
c) It has a water tight shape and offers greater resistance to pressure as compared to a clear corneal incision

Disadvantages are:

a) Greater possibility of ballooning of conjunctiva which can lead to poor visibility in AC

b) Increased risk of subconjuctival haemorrhage

Clear corneal incision

The incision is considered to be clear corneal when its external wound is positioned anterior to limbal vascular arcade (0.5mm anterior to limbus).

Advantages of clear corneal incision are :

a) It eliminates need to manipulate the conjunctiva

b) Aesthetically satisfactory result as eye does not appear to be operated

c) Intra-operatively tunnel is visible and manipulation of instruments in anterior chamber is easier

d) Requires less time and fewer instruments

Disadvantages:

a) Poor stability : Any incision more than 4 mm without suture may be unstable

b) Post operative endophthalmitis. There may be increased chance of endophthalmitis.

Clear corneal incision are invaluable in presence of filtering bleb, patients with coagulation disorders, patients with history of alcohol abuse and finally, while operating under topical anesthesia.

Position

With respect to corneal curvature, the incision can be positioned in:

a) Temporal quadrant

b) Superior quadrant

c) Obliquely (Superotemporal quadrant)

d) In axis of greatest curvature

The choice of position depends on two factors, induced astigmatism and the ergonomics of the operation. Until a few years ago, an incision in the superior quadrant was the most popular. It causes slightly more astigmatism (ATR) than its temporal counterpart.

Nowadays, this site is generally used for making sclero-corneal incision. Clear corneal temporal incision is the most favored incision with the majority of surgeons. It produces least or no astigmatism, easier access to surgical zone, good red reflex and it facilitates the drainage of irrigating solution.

Dimensions

The size of incision depends upon the size of lens to be implanted. It varies from 2.8 to 4 mm for foldable lenses and 5.5 to 6.5 for rigid lens. In foldable category, it also depends upon the size of phaco probe which may vary from 2.8 to 3.2 mm. Size of entry wound should match with size of phaco probe. Any incision in clear cornea more than 4 mm should be sutured.

Paracentesis (Side port)

Procedure starts with making a side port entry and injecting viscoelastic in the anterior chamber. Side port incision is made about 2 o'clock left of entry wound. It is made in clear cornea with 15° angled knife or 20 G MVR blade. The side port should measure about 1 mm and run parallel to iris plane. After supporting the globe by placing a toothed forceps outside limbus opposite to the site of making side port, enter the AC with 15° angled knife or MVR blade.

Construction of entry wound

Now make a vertical partial thickness incision at or inside the limbus. It should involve half to 2/3rd of corneal / limbal thickness. Now a 3 mm keratome is pushed into the depth of the wound and angled forward into the layers of the cornea for about 1.5mm.Direction of keratome should be forward and upward following the curve of cornea. Now the direction of keratome is changed downward to cut the Descemet's membrane and penetrate into the AC Once it has entered the AC, it returns to direction parallel to cornea to extend the entry to the required dimensions.

Capsulorrhexis

Capsulorrhexis, also known as Continuous curved capsulorrhexis (CCC) was developed independently in the mid 80s by Gimbel and Neuhann. This single innovation has made phacoemulsification a very safe procedure.

Techniques:

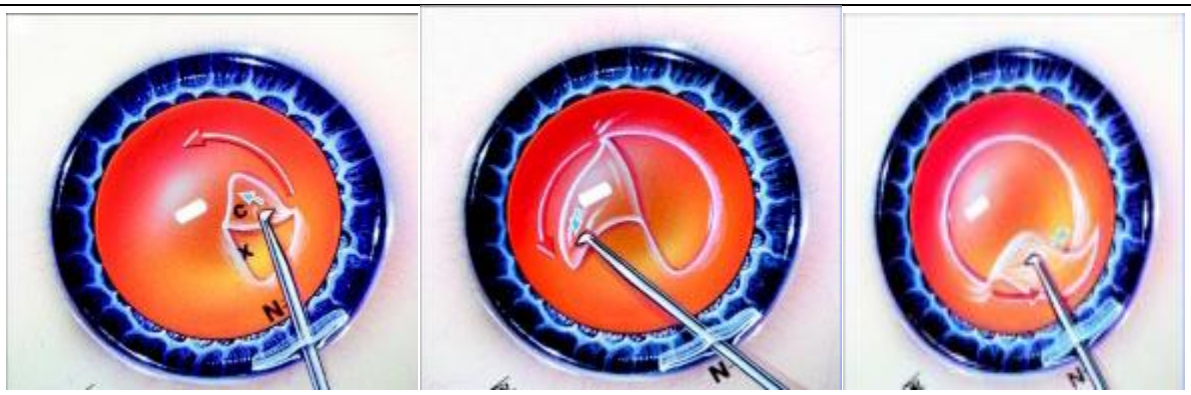
Capsulorrhexis can be performed with bent 26 or 27 G needle or capsulorrhexis forces (Utrata's forceps). It is remarkably easy with forceps. Fill the AC with viscoelastic. Start filling from 6 o'clock and withdraw syringe while injecting. A single perforation is created in anterior capsule at 12 o'clock position and extended radially for 2-3 mm to raise a triangular flap.

After a triangular flap has been raised and folded over itself (pushed over the anterior capsule), it is rotated clockwise. The needle is repositioned frequently, at least five to six times, always on upper surface of the flap and close to end of the previous sector. Certain precautions are necessary at this point.

1. AC should always be full with viscoelastic.
2. Do not go deep otherwise cortical matter will get disturbed, muffling the details inside the AC and making identification of the capsular flap difficult.
3. Once flap has been raised, the direction of force should be towards the centre.

Fig. 8

CAPSULORRHEXIS (CCC)



Capsulorrhexis procedure
Start with the capsulotome (N) in the centre, raise a flap (C), fold it on itself, rotate 360° to complete

Capsulorrhexis with Utrata forceps can be performed after raising a flap with needle as described above or an opening can be made in anterior capsule with forceps itself to raise a flap. Once the flap has been reversed, it is grasped with forceps and rotated clockwise or counter-clockwise. It should be regrasped again and again in more proximal position to maintain complete control. Make a 360° rotation and while finishing, bring it from outside to inside rather than inside to outside. It is desirable that AC must be reinflated again with viscoelastic if chamber collapses as it is likely to happen more with forceps.

Basic rules of capsulorrhexis

1. Always keep AC filled with viscoelastic
2. Use high magnification
3. Look for best red reflex
4. Be patient. Operate slowly and carefully
5. Repeat capsular grasping several times

Ideal capsulorrhexis is about 5-5.5 mm in size, well centered and circular. This will make future maneuvering safe and easy. If capsulorrhexis has extended into the periphery, it is better to convert to ECCE, especially for beginners. There is no need to prolapse the nucleus into the AC. Ensuring free rotation is however essential.

NUCLEUS MANAGEMENT

DIVIDE AND CONQUER

Divide and conquer nucleotomy incorporates 4 basic steps:
1. Sculpting until a very thin posterior plate of nucleus, if any remains
2. Fracturing the nuclear rim and posterior plate of the nucleus and nuclear rim
3. Fracturing again and breaking away a wedge shaped section of nuclear material for emulsification and
4. Rotating the nucleus for further fracturing and emulsification.

Crater Divide and Conquer (CDC)

Initially, deep central sculpting is done, resulting in a large crater, and leaving a dense peripheral rim to fracture into multiple sections. Once this is complete, the nuclear rim is fractured, using the bimanual method in which the spatula/chopper and the phacoemulsification tip create a counter pressure. The lens is rotated and a second crack is made, isolating a pie-shaped section. The nuclear rim is then rotated clockwise, for systematic piece-by-piece nucleofractis. The harder the nuclear rim, the smaller the wedge-shaped sections must be, to allow manageability and to reduce the possibility of tearing the posterior capsule.

Usually when performing CDC, especially in dense and brunescent cataracts, rather than immediately emulsifying each wedge-shaped section, the nuclear sections are left in place for capsular bag distension. Once the fracturing is complete each pie shaped wedge of the nuclear rim is brought to the centre of the capsule where phacoemulsification is safely accomplished. The ultrasonic turbulence is contained within the lens bag and absorbed by the lens, for all but the last one or two nuclear fragments.

Trench Divide and Conquer (TDC)

In soft lenses, after making a central trench a central fracture is created, and then the left as well as right sides of the lens are divided by fracturing.Many variations of this TDC technique exist and are used depending on density of the lens and the surgeon's choice. In softer cataracts, the firm nucleus is small and the epinucleus quite soft. The trench should be small, central and vertical in these nuclei to leave enough firm nucleus so that the force of two instruments can beapplied in nucleofractis. When reaching the 6.0 o'clock epinucleus, the phacoemulsification tip, by going deep to the anterior capsule rim, aspirates the epinucleus and peripheral cortex because of the adherent tendency of this material.

In soft nuclei, the lens is split by exerting lateral pressure with the phacoemulsification tip and spatula at the very center of the lens where the nuclear density is sufficient to resist cutting through the tissue with the instruments particularly with the spatula. With the lateral pressure at this point in the lens, the splitting usually starts in the posterior pole of the lens and extends towards the 6.0 o'clock positions for a complete splitting of the nucleus. The phacoemulsification tip is then used to impale the left-and-right-hand sections, and quadrants are fractured.

With increasing density of the lens it becomes more difficult to fracture the lens in this way unless the trench is made to its full length. This is accomplished by rotating the lens 180 degrees after sculpting is completed at the 6.0 o'clock position, so that the 12.0 o'clock area is now positioned at the 6.0 o'clock. For expediency, it is easier and more efficient to fracture the inferior rim with the phacoemulsification tip and spatula positioned just inferior to the centre of the lens.

After this initial fracture, which usually extends to one half or three quarters of the diameter of the lens, the fracture can be made complete by withdrawing the two instruments back to a position just superior to the center of the lens where lateral pressure will often then extend the fracture through the nucleus at the 12.00 O' clock position.

After the initial split inferiorly that extends to the centre of the lens or three quarters of the diameter, subsequent fracturing can be accomplished without the first fracture complete to the 12.0 o'clock position. The direction of the phacoemulsification tip is angled to the left and with a little more sculpting centrally the tip burrows deeply into the nucleus of the left hemisection. While the hemisection is stabilized with a spatula and aspiration, but no ultrasonic power the phacoemulsification tip is pushed and rotated clockwise to break off a pie-shaped inferior section of the lens. In softer nuclei this usually will break as a quarter section.

In more firm nuclei one can try to break away a smaller section, usually about one third of the hemisection. After this section is pulled to the centre by aspiration, it is emulsified.

Then, either another section can be similarly broken on the left hand side or the spatula can rotate the remaining three quarters of the nucleus counter clockwise, so that the phacoemulsification tip can then burrow into the right hemisection and break away one third or one half of this section while the remainder is stabilized with the spatula.

After fracturing is complete, the phacoemulsification setting is switched to the pulse mode to enhance holdability of the nuclear fragments to the phacoemulsification tip. The second instrument is then used to elevate the central apex of each quadrant and the phaco tip is used to deeply engage the nuclear fragment. Once occlusion has occurred the segment is brought into the middle of the epinuclear shell to be emulsified. The remaining quadrants are rotated and sequentially emulsified in a similar manner, leaving an intact epinuclear shell.

Four Quadrant divide and conquer

Beginner should start with sculpting. This is preferably done with 30° or 45° needle. Setting of machine should be as follows.

Vacuum : 20-40 mm Hg

Flow : 18-20CC/ minute

U/S power : 70%

After stabilizing the nucleus with spatula/ chopper introduced through side port, start making a groove on the nucleus from 12 to 6 o'clock. Press the foot pedal to position 3 and move the handpiece toward 6 o'clock edge of the capsulorrhexis. Never try to reach to the edge of the nucleus. Once at 6 o'clock, comeback to position 2 on foot pedal. The groove should be one phaco-tip wide and depth should be about 80%. Depth can be judged by depth of two sides of the groove and presence of red reflex obtained through the remaining nucleus. In soft nucleus, trench should be narrow deep and localized to central 2/3 whereas in hard cataract trench should be wide and reach further toward the edge of nucleus. Now, engaging the spatula at the end of tunnel rotate the nucleus by 90°.

Same procedure is repeated for each half. In between, that is, when rotating the nucleus, the foot pedal should be at position 1(irrigation only). After required depth has been achieved, stage is set for cracking the nucleus. With foot pedal at position 1, place the phaco-tip on right side and spatula or chopper on other side and separate the piece with lateral gentle force. It is very important that both instruments should be placed deep in the groove. A beginner can use two spatula/ choppers to divide the nucleus after filling the eye with viscoelastic. The direction of force should be horizontal with no vertical component. The procedure is then repeated to completely separate the four quadrants. At this stage, ensure the crack is complete posteriorly and all four quadrants are separated from each other.

Change the machine parameters as follows:
Vacuum: 150-200 cc
Flow: 20-24 cc/ minute
U/s power: 20%
Engage one piece at its depth, bring it forward and emulsify in iris plane.
Repeat same procedure for other 3 quadrants.

Phaco Chop

This technique is based on a principle that adopts the physics of splitting wood. A chopping instrument (thehatchet) is used to split the nucleus (the log) resting against the phacoemulsification tip (the chopping block). This permits the nucleus to be fractured along its longitudinal fibres using appositional forces rather than the parallel forces used by Gimbel. Every motion pulls the nucleus in towards the middle of the capsular bag, moving it out of the capsular fornix.

After completing capsulorrhexis and the hydrodissection, the phacoemulsification tip is placed in the eye burying it in the nucleus as far superiorly as possible. The nucleus is held firmly preventing it from moving superiorly as the chop is performed.

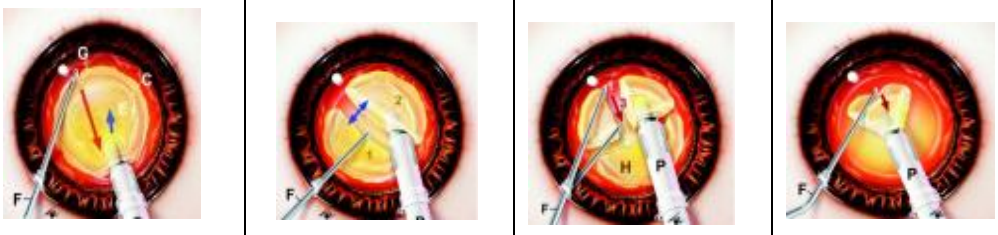
Next, an instrument, that is a chopper is placed in the eye through the side port incision and pushed down into the nucleus as far as the capsulotomy would allow. It is then pulled up towards the phacoemulsification tip, ripping a narrow groove in the nucleus as it cuts its way towards the chopping block.

As the second instrument (chopper) approaches the phacoemulsification tip the two instruments move gently apart, the chopper to the left and the phaco tip to the right effectively chopping the nucleus in two pieces. The operation continues, with the nucleus being rotated 90 degrees orienting the original chops horizontally and then the steps were repeated on the inferior half of the nucleus. The phacoemulsification tip is buried in the nucleus half just inside the original chop, the chopper is placed inferiorly and pulled up towards the phacotip, chopping the inferior nucleus half into quarters. After another rotation, the second nucleus half is also chopped into two. The four quarters were now emulsified.

Stop and Chop

Koch and Katzen, modified the phaco-chop technique to provide space for tissue separation, nucleus manipulation, and aid ease of removal. Without tissue removal the nuclear segments could fall back together after each chop, reassembling like an intraocular jigsaw puzzle. Hence a crater or a trench is made first and then one stops and then chopping is performed.

Fig.9 STOP& CHOP TECHNIQUE



Initial trench	Rotation & separation ofnuclear fragment	Chopping of each half	Phacoemulsification of nuclear pieces

Stop & Chop technique
G - Golden Ring; C - Cortex; F - Sharp chopper ; P - Phaco tip; H - Nuclear half;
1,2 - Breaking nucleus in two halves; 3 - Chopping the nuclear half

ASPIRATION AND IRRIGATION

The instruments used for irrigation aspiration can be automated or manual. Automated systems of I/A have the following advantages.

1. Vitreous is pushed back thus ensuring safety of posterior capsule.
2. Less chance of endothelial damage due to well maintained AC.
3. Easier I/A because of open and accessible capsular fornices.

Manual I/A however, has greater flexibility, greater surgeon control and is easily learnt.
Aspiration following phacoemulsification is different from ECCE. The amount of cortex after the former is much less, it is well hydrated and thus easily aspirated.

Further, there are no loose capsular tags and capsular fornices are well opened due to well maintained AC. However, it is difficult to remove the cortical matter from 12 o'clock region because approachability is limited due to CCC and small incision, especially when it is clear corneal.
The stages of adequate cortical clean up include.
1. Seeking occlusion
2. Grasping which implies a greater occlusion by activating suction and
3. Traction to strip the cortical tissue.
There are several difficulties in aspirating subincisional cortex.

Reasons for difficulty during aspiration of subincisional cortex with the tunnel incision

- Long pathway of the tip inside the tunnel
- Difficult verticalization of the tip as well as access to the capsular equator
- Increase in fluid leakage and decrease of chamber depth due to divarication of incision lips
- Small rhexis
- Distally decentralized rhexis
- Possible miosis
- Corneal folds

The methods described to aspirate the **subincisional cortex** are the following:

1. Post IOL implantation, dialing the IOL loosens cortex making aspiration easier.
2. Using a 26 G 'J' shaped cannula
3. Using a 30 G cannula through side port, i.e. suck and spit method. The entire lens matter is engaged using the cannula and pulled towards 6 o'clock and released into the anterior chamber. This is then aspirated using Simcoe I/A cannula or a syringe filled with 0.25 cc BSS.
4. 6 o'clock approach - A stab incision is given at 6o'clock and 30g cannula isused through the side port.

Vacuuming and polishing can also be done with I/A tip with suction kept at the minimum (25-30mmHg).

INTRAOCULAR LENS IMPLANTATION

The IOLs which can be used after phacoemulsification are single piece PMMAIOLs or foldable IOLs. The rigid IOLs used in phaco surgery have an optic diameter of 5.5mm or less so that only one suture is required to close the wound. Foldable IOLs can be made of soft **acrylic and hydrogels or silicon ematerial**. The design can be either **three piece lenses or single piece or platehaptic** design. Presently, three piece lenses are used and plate haptic design is used for toric IOLs to correct astigmatism of < 3D.

The **insertion devices** can be either a specially designed **forceps or injector** systems. The folding techniques are based on **horizontal principle** which allows a one step implantation or an**vertical principle**which requires a 2 step implantation. Silicone lenses cause less endothelial and uveal damage and are autoclavable. However, they may be associated with greater decentration and posterior capsule opacification (PCO) or after cataract as compared to acrylic lenses. Further, these cannot be implanted is eyes with silicone oil or where future vitreoretinal procedures are anticipated. As of now, the acrylic lenses provide the best visual performance and least amount of PCO.

PHACOEMULSIFICATION FOR A BEGINNER

Phacoemulsification has a long learning curve. Failures at every stage are bound to come. But at sometimes this must be learned and mastered. As this is technique for present and future and without phacoemulsification professional survival of cataract surgeon may be in danger. Here outlined are a few steps with Phacoemulsification procedure:

1. Be well conversant with use of microscope including use of foot control
2. Know your machine well
3. Select a case with patient age about 50-60 years, well dilated pupil, Grade-II / III nucleus hardness, and preferably right eye for right handed surgeon
4.Make a limbal bevelled incision at 12 o'clock or superotemporal site. So if need arises it may be extended
5. Ensure nucleus rotation by good hydro dissection
6. Perform a capsulorrhexis
Any failure at stage 4 (premature entry with frequent iris in section) and 5 &6 consider converting to ECCE.
7. Start with sculpting and four quadrant divide and conquer
8. Ensure first vertical crack is complete vertically and more important posteriorly Phacoemulsification is an ordinary human effort. If others can do it so can you. By determination and perseverance you will definitely be able to master it

What to expect with cataract surgery

Before surgery:

An ophthalmologist will measure affected eye to set the proper focusing power for our IOL. Also, the doctor will ask about any medicines currently used by the patient. Some of these medicines may have to be stopped before surgery. The prescribed eye drops are to start before surgery. These medicines help prevent infection and reduce swelling during and after surgery.

The day of surgery:

Cataract removal surgery may be done in an outpatient surgery center or in a hospital.

- The eye will be numbed with LA eye drops or with an injection around the eye.
- The patient will be awake during surgery seeing light and movement during the procedure, but not able to see what the doctor is doing to the eye.
- Surgeon will enter into the eye through tiny incisions (cuts, created by laser or a blade) near the edge of the cornea.

- The surgeon uses these incisions to reach the lens using very small instruments and will break up the lens with the cataract and remove it by motorized suction apparatus. Then the IOL is inserted into place.
- Usually surgeon will not need to stitch the incisions closed. These "self sealing" incisions eventually will close by themselves over time. A shield will be placed over the eye to protect it while it heals from surgery.
- The patient will be rested post-operatively in a recovery area for about 15–30 minutes. Then will be ready to go home.

POST SURGICAL RECOVERY:

Days or weeks after surgery:

- The patient must use eye drops after surgery, following the surgeon's directions for using these drops
- Should avoid getting soap or water directly into the eye
- Do not rub or press on the eye. An ophthalmologist may ask to wear eyeglasses or a shield to protect the eye
- The patient has to wear a protective eye shield while sleeping
- The ophthalmologist will talk about how active one can be soon after surgery. He/She will tell when one can safely exercise, drive or do other activities again

IOL Implants: Lens Replacement after Cataracts

An intraocular lens (or IOL) is a tiny, artificial lens for the eye. It replaces the eye's natural lens that is removed during cataract surgery. The lens refracts light rays that enter the eye, helping you to see. Our lens should be clear. But if we have a cataract, our lens has become cloudy. Things look blurry, hazy or less colourful with a cataract. Cataract surgery removes this cloudy lens and replaces it with a clear IOL to improve our vision.

IOLs come in different focusing powers, just like prescription eyeglasses or contact lenses. An ophthalmologist will measure the length of the eye and the curve of the cornea. These measurements are used to set the IOLs focusing power.

INTRAOCULAR LENSES (IOLs)

There are several choices of intraocular lenses used in cataract surgery – a standard monofocal intraocular lens (IOL), a toric IOL, a multifocal IOL or an accommodating lens:

A standard monofocal IOL is a fixed lens (it doesn't move) that is designed to deliver improved vision at one distance (usually far). The potential drawback is that after surgery, we may need to wear glasses for near and intermediate vision, even if, didn't wear glasses before surgery.

A toric IOL adjusts for individual astigmatisms and may minimize the need for distance vision glasses after surgery. The *TRULIGN* Toric IOL corrects the cataracts and astigmatism. It also gives a broader range of vision, from arm's length to distance.

A multifocal IOL is designed to deliver improved vision for distance and near. However, some patients may experience some halos and glare when driving at night, and some patients have difficulty adjusting to their new vision.

An accommodating IOL is designed to "flex" or "accommodate" using the eyes natural muscles to focus on subjects at various distances, delivering a fuller, more natural range of vision. *Crystalens* AO Lens was the first FDA-approved accommodating lens available in the United States. *Crystalens* is an artificial lens implant that, unlike a standard IOL, can treat both a person's cataracts and presbyopia—loss of near and intermediate vision. We probably noticed in our forties that we started to lose some of our up-close vision and had to start wearing reading glasses. Crystalens not only treats the cataracts but provides a more natural range of vision. It does so by recreating accommodation similar to our eye's natural lens. The unique Crystalens is designed to allow to enjoy most activities, including: reading a book, working on the computer, and driving a car.

Prevention of Cataracts

To reduce the risk of developing cataracts:

- Protect eyes from UVB rays by wearing sunglasses outside
- Have regular eye exams
- Stop smoking
- Eat fruits and vegetables that contain antioxidants
- Maintain a healthy weight
- Keep diabetes and other medical conditions in check

Latest trends

Another sophisticated piece of technology that is even newer is called ‘Intraoperative wavefront aberrometry’. Once the cataract has been removed, a device attached to a microscope measures the total refractive error of the eye. "This essentially allows us to more accurately ensure we're implanting the appropriate lens power for the eye, and increases our chances of hitting our target. This has been especially helpful for people who have had previous laser vision correction, such as LASIK or PRK, where the usual preoperative measurements and calculations are not as accurate.

The laser and ‘intraoperative wavefront aberrometry’ have also improved accuracy when eliminating astigmatism that causes images to appear distorted or blurry. During a cataract procedure, the surgeon can correct minor astigmatism by making carefully placed incisions in the cornea to normalize its curvature. For more severe astigmatism, the surgeon can insert special lenses into the eye, called toric intraocular lenses. "This allows a person to see more clearly at a distance with less dependency on glasses, though reading glasses are generally still required for near vision".

Actually, all standard replacement lenses require us to use reading glasses after surgery, regardless of whether have astigmatism. However, we can invest in special multifocal replacement lenses, similar to bifocal eye glasses, that correct both distance and close-up vision. Most people with extensive astigmatism aren't candidates for these lenses.

Hope on the Horizon: Stem Cells Treating Cataracts

Outside of first world countries like the U.S., surgery isn’t always an accessible option. There is however hope on the horizon. New cataract treatments will be less invasive; simplified surgical methods and cataract fighting eye drops could change the landscape for cataracts sufferers of the future.

Stem cells, which have been studied in the treatment of a variety of ailments, are also being researched in the cure of ocular issues. Scientists from China and the U.S., working in collaboration with one another, recently performed surgeries to remove the cloudy cataracts from the eyes of monkeys and rabbits. The lens capsule, however, was kept intact after the procedure, along with epithelial stem cells. In the experiment these stem cells went on to

regenerate the missing lens over the span of several months and vision subsequently increased. The consistently successful results produced in the animals allowed the test to be expanded to several human children, where the results were once again positive.

Additional testing needs to be done, as it is thought that for adults, stem cell treatment for cataracts could be more difficult as they have less responsive epithelial stem cells than younger patients. For children however, this innovation is especially exciting because they shouldn't need those subsequent surgeries that are currently standard with cataracts treatment.

Treatment of cataracts can occur as a result of other eye diseases, they mostly develop naturally with age. In fact, by age sixty five, many people will develop a cataract.

Literature Review

II. LITERATURE REVIEW

CATARACT:

Cataract is the single-largest factor for blindness in India, accounting for nearly 63 % of the total burden of vision impairment in the country.

It is reported that after cataract (62.7 per cent), in descending order of prevalence, the causes of blindness were uncorrected refractive error (19.7 per cent), glaucoma (5.8) per cent, posterior segment disorder (4.7 per cent) and corneal blindness (1 per cent).According to a group of ophthalmologists, who were speaking to the media at Rajendra Prasad Eye (RP) Centre for Ophthalmic Sciences at the AIIMS, India currently has the highest numbers of blind and visually impaired people.

"In India, there are nearly 0.8 crore blind and 5.4 crore visually impaired. Nearly 80-90 per cent blindness is avoidable and more than 90 per cent of it is seen in people aged 50 years and above," said the Chief of Rajendra Prasad (RP) Eye Centre for Ophthalmic Sciences at All India Institute of Medical Sciences (AIIMS), New Delhi.

Stating that patients were ignorant about various causes which damaged optic nerves a Professor of Ophthalmology at AIIMS, said that tobacco, betel nut and lime also caused damage to eyes, without the patients having any idea. Some medicaments, esp., Steroids are also a reason behind blindness. This is becoming common and nearly 20 per cent of children with blindness is because of the steroids. However, with the advancement in age, chances of people going blind due to steroids become less. The RP Centre is also conducting a "National Blindness Survey 2015-18" with a sample size of 90,000 people, using Rapid Assessment of Avoidable Blindness (RAAB) method.

Blindness survey 2015-18 is scheduled to be completed in 2018. Doctors said that the survey would provide the most reliable representative current estimates of blindness and visual impairment among those aged 50 and above. It will also generate, for the first time, the burden of diabetic retinopathy and sight threatening diabetic retinopathy in a representative community sample.

At the turn of the century, WHO and the International Agency for Prevention of Blindness launched the **Vision 2020**: the right to sight initiative **(**Foster A. 2000). The most recent estimates from WHO reveal that 47.8% of global blindness is due to cataract and in South Asia region which includes India, 51% of blindness is due to cataract **(**World Health Organization, 1997). Since cataract is a major cause of avoidable blindness in the developing countries, the key to the success of the Global Vision 2020: the right to sight initiative is a special effort to tackle cataract blindness **(**Foster A. 2001). Cataract surgery has been viewed as one of the most cost-effective health interventions with a cost of disability-adjusted life years saved of US $ 20-40.

Cataract surgical rate is a quantifiable measure of the delivery of cataract services in a country **(Limburg H *et al.*, 1997)**. It is thus a good indicator of how well a country is organizing its efforts in tackling cataract-related blindness. There has been a substantial increase in Cataract surgical rate (CSR) in India especially after the inception of the World Bank-supported Cataract Blindness Control Project **(Jose R and Bachani D. 1995)**. Professional interest and technological upgradation of skills and the availability of affordable equipment and intraocular lenses have all fuelled the increase in cataract surgery in India. A CSR of 3000 was targeted under Vision 2020: the right to sight, for India, by the year 2000. Current trends show that this target has been achieved, but still there are regional disparities across the country. The CSR for the year 2002-03 ranges from a high of 8440 per million populations to a low of 130 per million population**(Jose R and Bachani D. 2003)**.

According to **Jose R and Bachani D. (2003)** most of the bigger states in the country have already achieved a CSR of > 4000 per million population. The states of Gujarat (8440), Puducherry (7440), Tamil Nadu (5920) Andhra Pradesh (5260), Delhi (5090), Punjab (4950), Maharashtra (4840), Karnataka (4560) and Haryana (4180) have already gone past the recommended norm for Vision 2020: the right to sight. In view of this encourageing performance, it is very likely that the entire country can achieve a CSR of 6000 + per million population by 2020. This increased performance will reduce the prevalence of blindness and severe visual impairment in the country as half the blindness in India is attributable to cataract **(Murthy GV *et al.*, 2005).**

In epidemiologic parlance, a ′true′ rate is a proportion and is defined as the presence or absence of a characteristic in a group of people among people who are at risk of developing the particular disease during a specific time period. Therefore the denominator should comprise only individuals in the population who are at risk of developing the disease. In such a case, the denominator of the general population is not appropriate for defining CSR as the younger population would not be at risk of age-related cataract. It is therefore recommended that since most blinding cataract occurs after the age of 50 years, the denominator for CSR should consist only of the 50+ population.

If CSR per million 50+ population is considered, then by 2020, elimination of avoidable blindness due to cataract does not seem to be possible. In addition, if the incidence of new cataract blind individuals is added to the prevalent cases, it appears that India would be a long way off from eliminating avoidable blindness due to cataract by 2020.

If 30% continue to remain blind after cataract surgery, then the CSR/ million 50+ will need to increase by a third at different time periods. The situation gets compounded further if in addition to the above parameters, sight restoration is considered, and only 60% of all cataract surgeries are done on the blind, then the elimination of avoidable blindness due to cataract appears to be a dream in India unless a significant proportion of the 50+ have been ′prevented′ from going blind by operating at better visual acuity and by ensuring a better postoperative outcome.

Cataract surgical coverage (CSC) is an efficient indicator for planning as it provides information on what proportion of those needing surgery have been covered and therefore is a good indicator of the work remaining. However, CSC needs population-based surveys to provide information which is not easily available as against CSR which is most readily available. It is difficult to say if there is any over-reporting in cataract surgery when information is compiled at the national level. It is important to look at what proportion of cataract surgeries actually lead to a decrease in blind people (presenting vision < 20/200 in the better eye) after surgery compared to their preoperative status. Other researchers also consider that monitoring sight restoration rate is very important for planning at the national level **(Limburg H *et al.*, 1996)** and **(Lewallen S &Courtright P 2002)**. This is important because the Vision 2020:the right to sight approach is targeted towards the bilaterally blind.

Surgeries on people who have a presenting vision better than 20/200, surgeries on the second eyes, and surgeries on the unilaterally blind would not help in restoring vision though they have a role in preventing future blindness and should not be accounted for when monitoring progress towards the goal of elimination of avoidable blindness due to cataract. For the immediate future when a significant proportion of the 50+ are blind, the first priority should be given to restoring vision to those already blind.

Therefore, it would be more meaningful to monitor sight restoration in addition to CSR/ million population and CSR/ million 50+ population to monitor progress towards Vision 2020. Recent evidence in India suggests that the visual outcomes after cataract surgery are not very good in some regions wherein the operated people continue to remain blind after surgery. Poor visual outcome has been reported in 15-25% of eyes following cataract surgery **(Limburg H *et al.*, 1999)**. A study in southern India reported poor or very poor visual outcome after cataract surgery in 51.9% of the operated eyes **(Dandona L *et al.*, 1999)**.

Another study in northern India showed that one-third of the eyes which had a preoperative vision of less than 20/200 continued to have vision less than 20/200 with best correction after cataract surgery **(Murthy GV *et al.*, 2001)**. A study in Mysore, India, demonstrated that more than one-third were blind in the operated eye **(Singh AJ *et al.*, 2000)**. Improving the quality of surgery is a major input that needs to be emphasized now that the quantity of surgery has been increased. This along with improved Sight Restoration Rate per Year(SRR) will be more effective in eliminating cataract blindness.

The projections have been made using a simple dynamic model using Microsoft Excel software considering assumptions as mentioned. If the assumptions change, then the projections would also change. Age-specific mortality has been used to project future population in different age cohorts and it is assumed that most of the surgical patterns and outcomes will remain constant to a large extent.

Most literature concentrates on the prevalence of cataract blindness in projecting future trends. However it is also important to consider the newly blinded individuals (incident cases of cataract blindness) as they would also need to be treated. The backlog of cataract blindness can be tackled effectively only if the incident cases are also accounted for. Unfortunately,

estimates of incidence of cataract blindness are difficult to obtain because of the long duration of the disease and the uncertainty of the pace of progression to blindness in cataract. Conventionally, it has been stated that the incidence of cataract blindness is 20% of prevalence **(Foster A. 1993)** and **(Duerksen R *et al.*, 2003).**

Studies in South Africa and India have documented that the incidence ranges between 23-30% of prevalence **(BhattacharjeeJ *et al.*, 1996)**. Assuming that the incidence of newly blinded cataract is 20%, it appears that the present number of cataract surgeries need to be scaled up significantly if the elimination of avoidable cataract blindness is to be a reality by 2020. It needs to be emphasized that outcome measures like CSR and CSR 50+, by themselves are inadequate to describe the benefit to the operated individuals and their quality of life. More widespread use of indicators like SRR and developing sensitive indicators for assessment of visual function after surgery should be effectively used in the future. At the same time, other causes of avoidable blindness need to be given adequate attention.

As may be evident about 10% of the cataract surgeries are done on patients <50 years of age and this trend may increase with increased incidence of diabetes, increase in posterior subcapsular cataract and improved phacoemulsification surgery penetration in India. More number of people would be operated upon sooner than they are today. Data from three surveys conducted in India show this clearly. Different methodologies were used in these surveys, and any inappropriate estimation in these surveys could affect the comparability of their findings in this study. The CSR and SRR suffer from the fact that they are based on reported figures whose validity or accuracy cannot be scrutinized. Some program managers suggest that the incidence of cataract in India has been overestimated **(Thomas R and Muliyil J 1998)**. If this is true, it may be easier to achieve the targets for elimination of avoidable blindness due to cataract in India as would improve visual outcomes after cataract surgery which is eminently feasible in the immediate future.

Although modern cataract surgery is extremely safe, postoperative endophthalmitis remains the most feared complication, and cystoid macular edema (CME) continues to be the most common cause of vision loss after routine cataract procedure. In 2013, discussion continued about the role of intracameral versus topical antibiotics for infection prophylaxis, and cataract surgeons had several new options for use in controlling inflammation after cataract surgery.

When it comes to the debate about endophthalmitis prophylaxis Regimens, a key event that year was the publication of a study by **Shorstein *et al.*,(2013)** from Kaiser Permanente Northern California that showed the rate of postoperative infections decreased after the institution of intracameral antibiotic use.

The intracameral use of antibiotics following cataract surgery significantly reduces the risk of postoperative endophthalmitis when compared to the pre-or postoperative use of topical antibiotics. The prophylactic use of this therapeutic Regimen is a current practice in Europe and in a large number of countries around the world. It is not routinely followed in the US because no commercial antibiotic for intracameral use has been approved by the Food and Drug Administration. With this update on the topic, the Brazilian Society for Cataract and Refractive Surgery intends to show the positive impact of the intracameral use of antibiotics on reducing the rates of endophthalmitis following cataract surgery and highlights evidence of good outcomes that reinforce the safety and efficacy of this prophylactic therapeutic Regimen.

Herrinton *et al.*,(**2016)** performed a longitudinal, observational, and controlled cohort study of approximately 315,000 phacoemulsification procedures to identify which prophylactic Regimen was the most effective. They compared the intracameral use of antibiotics (cefuroxime or moxifloxacin) to the topical use of antibiotics (gatifloxacin, ofloxacin, polymyxin-trimethoprim, moxifloxacin, neomycin, gentamicin, or tobramycin). The authors found 215 cases of endophthalmitis (0.07% or 0.7 per 1,000 cases). The intracameral use of antibiotics was more effective than the topical use of a single antibiotic [OR: 0.58; confidence interval (CI): 0.38–0.91]. The combination of intracameral antibiotics and topical applications of gatifloxacin or moxifloxacin was not more effective than the intracameral use alone (OR: 1.63; CI: 0.48–5.47). When compared to the topical use of other antibiotics, topical gatifloxacin reduced the risk of endophthalmitis by 42%; neomycin, gentamicin, and tobramycin were less effective (OR: 1.97; CI: 1.17–3.31). The risk of endophthalmitis in patients not administered with intracameral antibiotics in this study was lower than that in other studies (0.044% risk of endophthalmitis in the intracameral group and 0.070% risk in the topical group). These data were consistent with those of the ESCRS study (0.05% risk of endophthalmitis in the intracameral group and 0.35% risk in the control group) **ESCRS (2007)**. The authors concluded that the intracameral use of antibiotics was more effective for

preventing endophthalmitis following cataract surgery than the topical use of antibiotics alone **(Arbisser LB. 2008).**

The topical use of antibiotics did not render the intracameral Regimen any more effective. The authors found no convincing evidence of a difference in efficacy between intracameral moxifloxacin and cefuroxime. In this study, moxifloxacin was used directly and concentrated or diluted in equal parts of balanced saline solution to obtain a concentration of 250 µg per 0.1 ml. The authors reported the intracameral application of cefuroxime in 13 eyes (11 patients), with an error of formulation of 9 mg, which resulted in acute macular edema that was resolved within 1 week with no further complications**(Yoeruek E. *et al.,* 2008)**. That was the first study to report an association between the intracameral use of cefuroxime and adverse effects. Increased resistance to fluoroquinolones may support the use of cefuroxime, particularly in patients exposed to fluoroquinolones in the past. The intracameral injection of any antibiotics may be less subject to the occurrence of resistance as the antibiotics are administered as a single concentrated dose in a relatively confined space in contrast to the topical use of antibiotics, which include repeated and less effective low doses of the drug **(Arbisser LB. 2008).**Vancomycin has also been used in intracameral injections to prevent infection and has been found to be effective against gram-positive bacteria. However, concerns over resistance have limited its use to approximately 1% of patients who are allergic both to penicillin/cephalosporins and fluoroquinolones. In their conclusion, the authors recommended the intracameral injection of cefuroxime or moxifloxacin in all phaco-emulsification surgeries. The use of a topicalagent alone is less effective and is also subject to errors in prescription and a lack of patient compliance. Topical aminoglycoside antibiotics are less effective in preventing endophthalmitis **(Hui M.*et al.,* 2011).**

Based on the results of approximately 3 million cataract surgeries in the US annually, the adoption of the intracameral use of antibiotics may potentially save more than 2,000 eyes per year from the negative impact of postoperative endophthalmitis **(Javitt JC 2016)**.

The intracameral use of antibiotics to prevent infection following cataract surgery and other intraocular surgeries began in 2002**(Montan PG *etal.,* 2002).** The multicenter, prospective, randomized study by ESCRS **(2007)** which evaluated the prophylactic use of intracameral

versus topical antibiotics, considered 16,000 patients and found 4.92 times more risk of endophthalmitis with the topical use of antibiotics. This result made the intracameral use of antibiotics widespread in Europe despite the lack of an approved intracameral antibiotic formulation. Later, the commercial formulation of cefuroxime for intraocular use was accepted across the European Union. It is important to note that the fixed price of cefuroxime was substantially lower than that of antibiotic eye drops**(Javitt JC 2016).**

The main limitation of the intracameral use of antibiotics in the US is the lack of approval by FDA of a commercial antibiotic for intracameral use. American ophthalmologists are dissuaded from the intracameral use of antibiotics based on the risk of legal and financial consequences because the product is not approved for this use and would therefore need to be used off-label. As previously mentioned, the preparation of the product in the operating room has many risks. In the US, despite the existence of pharmacies licensed to supply sterile products, they are requested only for special patients. Recently, FDA inspected 28 pharmacies authorized to produce sterile medications and identified violations in each one of them **(USFDA2013). Javitt JC (2016)** also reported the following barriers: a) no commercial sponsor has presented FDA with a new drug application, b) minimum financial return is expected, and c) there is a need for guidelines to regulate controlled safety and efficacy studies of the actual drug that the manufacturer plans to sell.

TOPICAL Vs. INTRACAMERAL ANTIBIOTICS:

Antibiotics

Antibiotics are drugs, either of synthetic or natural origin, that are used to treat infections caused by bacteria and other microorganisms. The discovery of antibiotics beginning with sulfonamides and β-lactams in the mid-20th century enabled rapid treatment of bacterial infections that had previously proven to be fatal. Between 1930 and 1970 in the “golden age” of antibiotic research, many classes of antibiotics that are still in use today were discovered. Since then, overuse and misuse of antibiotics has resulted in the emergence of antibiotic resistant microorganisms and has fuelled the continuance of antibiotic development. Increasingly, antibiotic resistance has become a major threat to public health. The various classes of antibiotics are active against different types of bacteria and can have either intracellular or extracellular targets.

Bacteria are generally classified into one of two groups based on the structural differences in their cell walls: Gram-positive or Gram-negative. Gram-positive bacteria have cell walls that are composed of a single peptidoglycan layer attached to an inner cytoplasmic membrane. Gram-positive bacteria retain crystal violet dye in the Gram staining protocol. In contrast, Gram-negative bacteria do not retain the dye. Gram-negative bacteria have an additional, second outer membrane, which acts as a permeability barrier and convers the peptidoglycan of the cell wall. Due to the additional cell membrane, it is more difficult for antibiotics to penetrate Gram-negative bacteria. Thus in many cases, classes of drugs that are useful against Gram-negative organisms are also useful against Gram-positive, but not vice versa. Narrow spectrum agents are active against only a specific type of bacteria, while broad spectrum agents are active against many different bacteria, often both Gram-positive and Gram-negative organisms.

Some antibiotics have cellular targets that do not require the antibiotic to fully transverse into the bacterial cell or cytoplasm, such as vancomycin that targets the peptidoglycan of Gram-positive bacteria, while others have cytoplasmic targets, such as sulfonamides and Fluoroquinolones. The activity of an antibiotic is commonly characterized by minimum inhibitory concentration (MIC). MIC is ascertained by a standard growth assay in which the concentration of the drug required to block visible growth of a bacterial population is determined. Lower MICs correspond to increased activity. MIC is a measure of bacterial growth and does not differentiate bactericidal and bacteriostatic antibiotics. Percent DNA cleavage is a measure of activity that is commonly used to evaluate activity of topoisomerase inhibitors, such as fluoroquinolone antibiotics. Higher percent DNA cleavage corresponds to increased activity. In the present work, both MIC and percent DNA cleavage are used as measures of fluoroquinolone-class antibiotic activity. Drug resistant microorganisms are responsible for an increasing number of community-acquired and hospital-acquired infections, including those from *Staphylococcus aereus*, *Pseudomonas aureginosa*, *Streptococcus pneumoniae*, and *Mycobacterium tuberculosis*. These drug resistant microorganisms display reduced or nonexistent susceptibility to antibiotic drugs, thus allowing the infections to persist in patients and increase the numbers of fatalities. When first choice antibiotics do not work to treat an infection, a second or third, often more toxic “drug of last resort” is administered in an attempt to treat the drug resistant infection. Consequently, usage restrictions have been placed on these drugs of last resort in order to decrease the chance of selecting for microorganisms that are resistant to these agents.

There are three general mechanisms of resistance that are common to many classes of antibiotics: chemical modification or breakdown of the antibiotic, failure of penetration including efflux transporters, or specific changes to the target. Common examples of chemical modification and antibiotic breakdown are the enzymatic addition of acyl functional groups to the aminoglycoside antibiotics, and the destruction of β- lactam antibiotics by β-lactamases. Failure of the drug to penetrate the microorganism can occur by several methods: mutation or down regulation of the drug transporters that take in the drug, upregulation of efflux transports that actively pump the drug out of the bacterium once it is inside, or alteration of the cell wall composition so the drug can no longer penetrate the bacterium.

Antibiotic targets can be altered through changes in expression levels or through spontaneous mutation in the genes encoding the target that cause alterations in target structure and prevent the antibiotic from interacting with the target. Alteration of the target through mutation is one of the main mechanisms of resistance to Fluoroquinolones, the class of antibiotics that is the focus of this dissertation. Each of these mechanisms of resistance prevents the antibiotic from effectively acting on its bacterial target. Resistance can exist, be selected for, and be promoted by using antibiotics. In a large population of bacteria, it is likely that there are several cells that are inherently resistant to one or more antibiotic agents due to the genetic variation within the population. When an antibiotic is applied to the population of bacteria, all the susceptible cells die, leaving behind the resistant bacteria. The remaining bacteria can then reproduce to produce a large colony of drug resistant microorganisms over a short period of time.

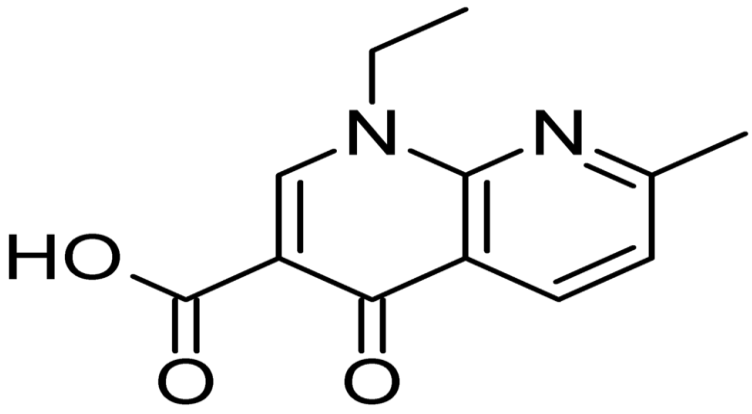
Current practice methods are unclear as to the most safe and effective prophylactic pharmacotherapy and method of delivery to reduce postoperative endophthalmitis occurrence. In a recent meta analysis it is stated that Intracameral antibiotics (cefuroxime and moxifloxacin) reduced endophthalmitis rates compared with controls with minimal or no toxicity events at standard doses. Additionally, intracameral antibiotics alone may be as effective as intracameral plus topical antibiotics **(Bowen R.C. *et al.*, 2018).**

Fluoroquinolone Antibiotics:

Fluoroquinolones are a class of broad spectrum antibiotics. Clinically, fluoroquinolone class agents have been used to treat a variety of infections due to their broad spectrum activity against Gram-positive and Gram-negative bacteria: respiratory infections, sexually transmitted diseases, and enteric infections among others. Fluoroquinolones are structurally based on nalidixic acid, an antibiotic of synthetic origin that was discovered in 1962 . At low concentrations, nalidixic acid was found to be bacteriostatic and inhibit bacterial cell growth, while at higher concentrations it is bactericidal, killing bacterial cells. However, at the time, the use of nalidixic acid was limited due to its narrow-spectrum activity against Gram-negative microorganisms. Thus, there was a need for nalidixic acid to undergo structural modification in order to attempt to improve its activity against a broader spectrum of microorganisms and therefore improve its clinical usefulness.

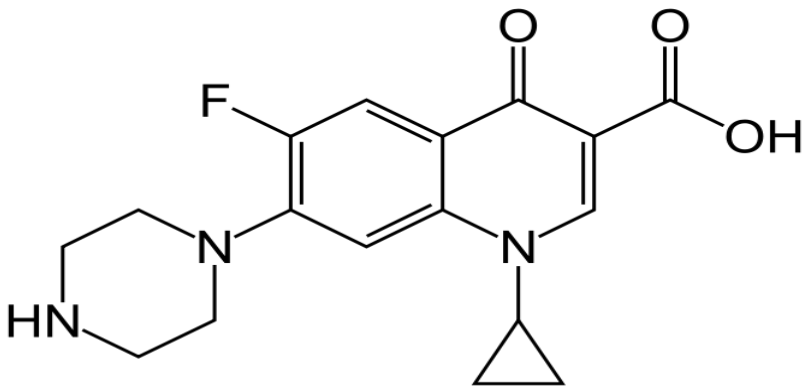
Since the synthesis of nalidixic acid and discovery of its unique antimicrobial activity in the early 1960's, fluoroquinolone class antibiotics have undergone four generations of clinical development. As in most antibiotic taxonomies, each generation has both structural characteristics and activity profiles that set it apart from other Fluoroquinolone structures by generation. The structures of nalidixic acid and select Fluoroquinolones from different generations of development are shown in the figures given below. Nalidixic acid and related acids were considered to be **first generation quinolone-class agents**. Structurally, nalidixic acid is a naphthyridone, not a quinolone, because its bicyclic core structure consists of two nitrogen atoms instead of one as in a quinolone core.

The First Generation Quinolone - Nalidixic Acid



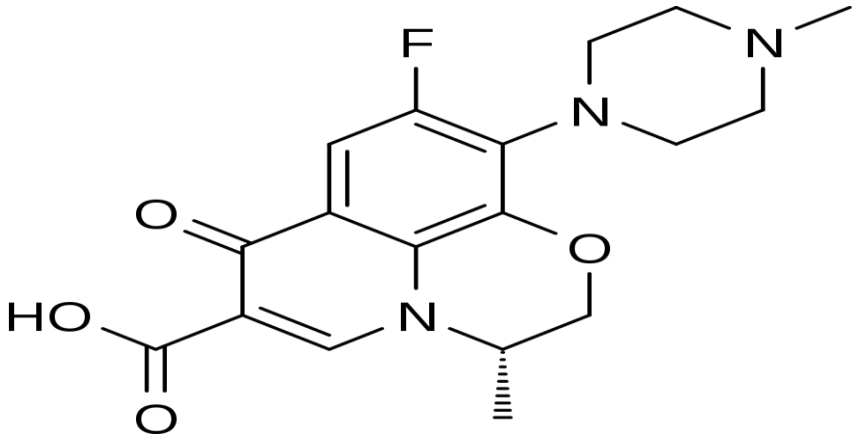
The switch to the quinolone core and introduction of a fluorine atom at C-6 and piperazine at C-7 in **second generation derivatives**, such as ciprofloxacin and norfloxacin, lead to increased spectrum activity and thus a larger range of infections that these agents could be used to treat. Addition of the N-1 cyclopropyl group, such as that in ciprofloxacin, was also found to be an important modification in increasing antibacterial activity.

The Second Generation - Ciprofloxacin



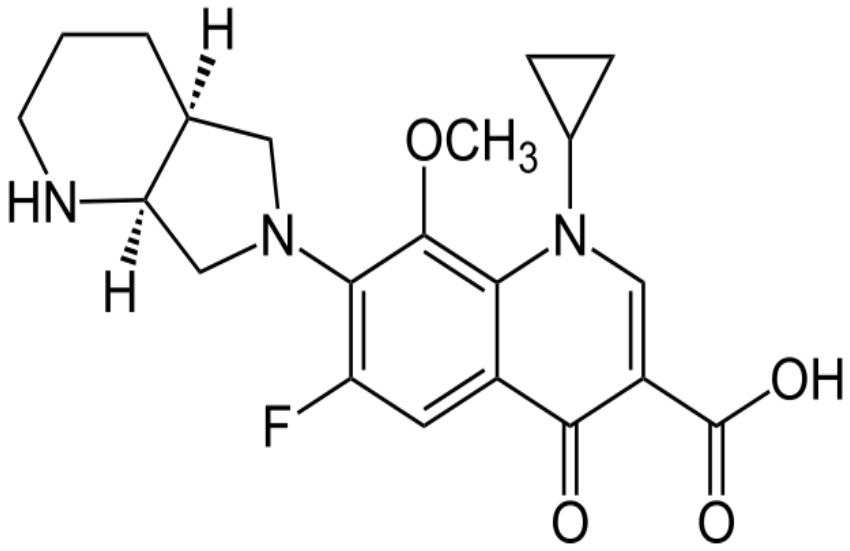
Further development of second generation derivatives with the introduction of new C-7 rings or chiral resolution of existing agents lead to **third generation derivatives**, such as levofloxacin. Third generation derivatives have demonstrated activity against Gram-positive bacteria and some penicillin resistant bacteria.

The Third Generation - Levofloxacin

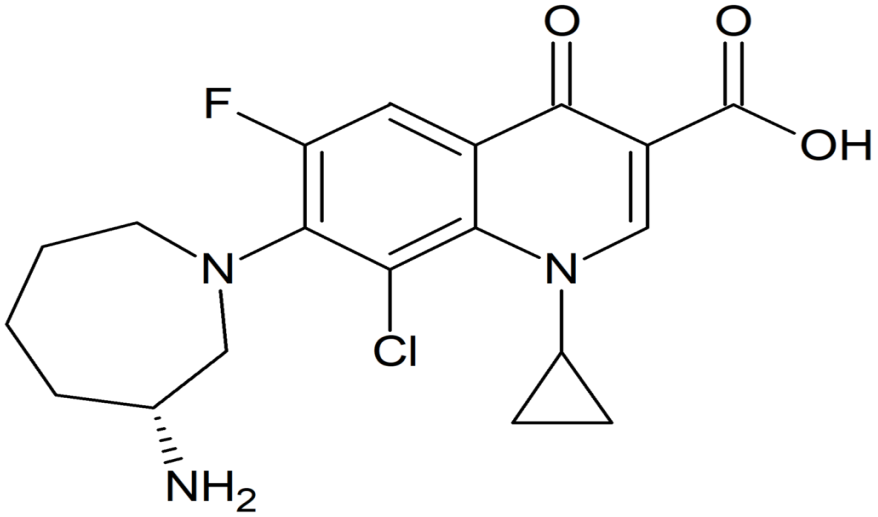


The C-8 methoxy functionality was incorporated into the structures of **current fourth generation** fluoroquinolone agents, such as moxifloxacin and besifloxacin. The C-8 methoxy functionality is thought to contribute to increased activity against Gram-positive microorganisms, as well as decreased phototoxicity, a problem that was seen with some earlier agents. These agents demonstrate activity against anaerobic targets and some ciprofloxacin (a second generation fluoroquinolone) resistant targets. When looking at the history of fluoroquinolone development, important trends are observed that make Fluoroquinolones an important class of antibiotics for the future.

The Fourth Generation - Moxifloxacin



The Fourth Generation - Besifloxacin



First, researchers are able to modify the structure of the fluoroquinolone to improve effectiveness against different kinds of microorganisms. While early generation Fluoroquinolones were effective against Gram-negative bacteria, the newer generation Fluoroquinolones have been developed to be more effective against Gram-positive bacteria. Additionally, researchers have successfully been able to modify the basic fluoroquinolone structure to gain antibiotic activity against bacterial mutants that arise and cause resistance. In the future, Fluoroquinolones could continue to undergo structural modifications in order to overcome the challenges faced by the on-going emergence of resistant microorganisms.

Role of fluoroquinolones

The ideal antibiotic agent, which could be used in conjunction with povidone-iodine, would have the four "killer Bs":

- Broad spectrum of activity
- Bactericidal mechanism of action
- Biocompatibility and
- Bioavailability

Bioavailability is especially important for antibiotic prophylaxis with cataract surgery; to be effective, an agent must penetrate the aqueous and, preferably, the vitreous humor.

For this reason, fluoroquinolones have emerged in Europe and the United States as the mainstay of topical antibiotic prophylaxis. The two current generation ophthalmic 8-methoxy fluoroquinolones, available in the United States, moxifloxacin and gatifloxacin, are particularly active against gram-positive species while also maintaining an excellent gram-negative effect. These agents are effective against endophthalmitis isolates that were resistant to the earlier generation fluoroquinolones ciprofloxacin, ofloxacin and levofloxacin **(Mather R *et al.*, 2002)**. The current generation fluoroquinolones are more efficacious against organisms that cause endophthalmitis in humans, notably *S epidermidis*, *S aureus* and *S pneumoniae*, than previous generations.

Increased resistance to fluoroquinolones has been reported in keratitis, conjunctivitis and endophthalmitis isolates**(Mah F.2003).** Increasing antimicrobial resistance has been reported worldwide**(Smith RD. 2002)**.

Studies of ocular pathogens indicate many bacteria now have resistance to multiple types of drugs, including fluoroquinolones, cephalosporins and methicillin. In a 2001 study, none of the endophthalmitis isolates were susceptible to the older fluoroquinolones (**Kowalski RP. 2003).**

A study by Blondeau and colleagues showed that the current generation fluoroquinolone gatifloxacin with benzalkonium chloride (BAK) has lower minimum inhibitory concentrations against a number of organisms than gatifloxacin without BAK. The combination of agent plus BAK was more effective than the agent alone; all clinical specimens of *S pneumoniae* tested, as well as a number of other strains, had lower MICs for the gatifloxacin solution with BAK than the drug without BAK.

A similar study showed that the topical agent gatifloxacin 0.3% with BAK had a faster kill curve than the fluoroquinolone agent moxifloxacin 0.5% alone against several strains of *Staphylococcus*. The strains were inoculated to the two antibiotics, and serially diluted samples were incubated for 72 hours at 35° C. Test samples were assayed at 15, 30 and 60 minutes.

Gatifloxacin 0.3% killed the bacterial inoculum completely at 30 minutes in 16 of 17 test strains. At 60 minutes, no bacterial colony was recovered from topical gatifloxacin 0.3% in all strains. Moxifloxacin 0.5% reduced the bacterial inoculum by 60 minutes in all strains, but complete killing within 60 minutes was observed in only one strain.

Bioavailability

The bioavailability of an antibiotic can be assessed on the basis of how high a concentration it reaches in the tears, the cornea or other ocular tissues. Topical administration results in significant concentrations achieved in the tear film and on the cilia and meibomian glands. Penetration into the cornea can also be achieved, with subsequent distribution providing potentially therapeutic levels in the aqueous humor.

Solomon and colleagues compared aqueous humor concentrations of two topically administered current generation fluoroquinolones and found that moxifloxacin achieved roughly twice the concentration of gatifloxacin in the aqueous humor in one study **(Solomon R *et al.,* 2009)**. Additionally, moxifloxacin achieved a maximum concentration (Cmax) of 10 times more than the MICs of most endophthalmitis-causing organisms **(Solomon R *et al.,* 2009)**.

A potentially protective concentration of moxifloxacin can be obtained in the aqueous humor of human patients with topical dosing prior to cataract surgery **(Kim DH*et al.*,2005).** Aqueous humor concentrations of 1.8 µg/mL for moxifloxacin and 0.48 µg/mL for gatifloxacin were achieved. The Cmax of moxifloxacin exceeded the known MICs of the organisms that most frequently caused endophthalmitis **(Mah F. 2003).**

Topical administration of a current generation fluoroquinolone may prevent endophthalmitis even with a huge inoculum. In one animal study, one drop of moxifloxacin or saline solution was administered at 60, 45, 30 and 15 minutes before injection of a broth containing 0.025 mL (5 × 104 colony-forming units) of *S aureus* into the anterior chamber of rabbits. After injection, four drops of moxifloxacin or saline solution were administered over 24 hours. At an examination at 24 hours, the moxifloxacin-treated rabbits did not develop clinical signs of endophthalmitis, while a large proportion of animals in the saline-treated group had signs of endophthalmitis (**Kowalski RP*et al.*, 2005)**.

Once an organism reaches the vitreous, topical application of antibiotics is probably not efficacious to prevent infection. Costello and colleagues concluded that penetration of topically applied moxifloxacin and gatifloxacin into the vitreous in the uninflamed eye was not at significant levels to be protective against endophthalmitis**(Costello P*et al.*, 2006)**.

Besifloxacin is a new fluoroquinolone anti-infective developed for ophthalmic use, Theophthalmic suspension 0.6% (Besivance™) was recently approved for the treatment of bacterial conjunctivitis. The objective of this article by **Proksch *et al.*, (2009)** is to provide a comprehensive overview of microbiological, pharmacokinetic/pharmacodynamic and clinical studies with besifloxacin. Microbiological studies have demonstrated that besifloxacin has wide-spectrum and potent activity against common ocular pathogens, including Gram-negative and Gram-positive pathogens associated with bacterial conjunctivitis, and retained activity against fluoroquinolone-resistant staphylococci and multidrug-resistant strains. In preclinical and human studies, topically applied besifloxacin had a prolonged ocular concentration and minimal systemic exposure. In clinical studies, patients randomized to besifloxacin ophthalmic suspension 0.6% experienced significantly higher rates of clinical resolution and microbial eradication than patients randomized to vehicle. Besifloxacin ophthalmic suspension 0.6% was also found to be as effective and well tolerated as moxifloxacin ophthalmic solution 0.5%. The low minimum inhibitory concentrations and high attainment of pharmacodynamic targets with besifloxacin may contribute to a lower risk

for the emergence of bacterial resistance, although further studies are needed. These data indicate that besifloxacin ophthalmic suspension 0.6% is an important new option for the treatment of bacterial conjunctivitis.

The primary objective of a study by **Karpecki P*et al.*,(2009)** was to compare the clinical and microbiologic efficacy of besifloxacin ophthalmic suspension 0.6% with that of vehicle (the formulation without besifloxacin) in the treatment of bacterial conjunctivitis. This was a multicenter, prospective, randomized, double-masked, vehicle-controlled, parallel-group study in patients with acute bacterial conjunctivitis. Patients received either topical besifloxacin ophthalmic suspension or vehicle administered 3 times daily for 5 days. At study entry and on days 4 and 8 (visits 2 and 3), a clinical assessment of ocular signs and symptoms was performed in both eyes, as well as pinhole visual acuity testing, biomicroscopy, and culture of the infected eye(s). An ophthalmoscopic examination was performed at study entry and on day 8. And the conclusion of this study is the Besifloxacin ophthalmic suspension 0.6% given 3 times daily for 5 days was both efficacious and well tolerated compared with vehicle in the treatment of these patients with bacterial conjunctivitis.

McDonald MB*et al.*,(2009) compared the clinical and antimicrobial efficacy of besifloxacin ophthalmic suspension 0.6% with that of moxifloxacin ophthalmic solution 0.5% for the treatment of bacterial conjunctivitis. Multicenter, randomized, double-masked, parallel-group, active-controlled, noninferiority study. A total of 1161 patients (533 with culture-confirmed bacterial conjunctivitis) were randomized. Based on the 95% confidence interval (CI) of the difference, besifloxacin was noninferior to moxifloxacin for clinical resolution on day 5 (58.3% vs. 59.4%, respectively; 95% CI, -9.48 to 7.29) and day 8 (84.5% vs. 84.0%, respectively, 95% CI, -5.6% to 6.75%) and for microbial eradication on day 5 (93.3% vs. 91.1%, respectively, 95% CI, -2.44 to 6.74) and day 8 (87.3% vs. 84.7%; 95% CI, -3.32 to 8.53).

There was no statistically significant difference between the 2 treatment groups for either efficacy end points on days 5 or 8 ($P>0.05$). Besifloxacin ophthalmic suspension was non inferior to moxifloxacin ophthalmic suspension and provided similar safety and efficacy (clinical and microbiological) outcomes when used for the treatment of bacterial conjunctivitis

Cambau E. *et al.*,(2009) Besifloxacin is a new fluoroquinolone in development for ocular use. They investigated its mode of action and resistance in two major ocular pathogens, *Streptococcus pneumoniae* and *Staphylococcus aureus*, and in the reference species *Escherichia coli*. Primary and secondary targets of besifloxacin were evaluated by: (i) mutant selection experiments; (ii) MIC testing of defined topoisomerase mutants; and (iii) inhibition and cleavable complex assays with purified *S. pneumoniae* and *E. coli* DNA gyrase and topoisomerase IV enzymes.

Although mutant selection experiments indicated that gyrase is a primary target, further biochemical and genetic studies showed that besifloxacin has potent, relatively balanced activity against both essential DNA gyrase and topoisomerase IV targets in *S. aureus* and *S. pneumoniae.*

Comstock TL*et al.*,(2010)examined the efficacy and safety of besifloxacin ophthalmic suspension 0.6% in patients aged 1-17 years with bacterial conjunctivitis. This was a post hoc analysis of a subgroup of pediatric patients aged 1-17 years who had participated in three previously reported, randomized, double-masked, parallel-group, multicenter, clinical trials evaluating the safety and efficacy of besifloxacin in the treatment of bacterial conjunctivitis.

This analysis included 815 pediatric patients aged 1-17 years (447 with culture-confirmed bacterial conjunctivitis). Clinical resolution was significantly greater ($p < 0.05$) in the besifloxacin group than in the vehicle group at both visit 2 (53.7% vs 41.3%) and visit 3 (88.1% vs 73.0%). Similarly, microbial eradication was significantly higher with besifloxacin than with vehicle at visit 2 (85.8% vs 56.3%) and visit 3 (82.8% vs 68.3%). No significant differences in clinical resolution and microbial eradication were noted between besifloxacin and moxifloxacin. Besifloxacin was well tolerated, with similar incidences of adverse events in the besifloxacin, vehicle, and moxifloxacin groups.

They concluded that Besifloxacin ophthalmic suspension 0.6% was shown to be safe and effective for the treatment of bacterial conjunctivitis in children and adolescents aged 1-17 years.

The study about Human aqueous humor concentrations of besifloxacin, moxifloxacin, and gatifloxacin after topical ocular application was carried out by **Donnenfeld ED*et al.*,(2011)**.

To assess these concentrations relative to the minimum inhibitory concentration for 90% of strains (MIC(90)) for each drug against bacterial pathogens identified in recent cases of postoperative endophthalmitis.

The aqueous humour drug concentrations were compared 60 minutes ± 5 minutes after instillation of 1 topical drop to patients aged 18 years or older having uncomplicated cataract surgery. Concentrations were determined using a validated liquid chromatography with tandem mass spectrometry method. Based on the aqueous humor drug concentrations measured in this study, it is unlikely that any of the fluoroquinolones tested would be therapeutically effective in the aqueous humour against the most frequently identified drug-resistant staphylococcal isolates from recent cases of postoperative endophthalmitis.

Silverstein BE*et al*.,(2011) has done the study evaluated the efficacy and tolerability of besifloxacin ophthalmic suspension 0.6% administered twice daily for 3 days compared with vehicle (formulation without besifloxacin) in the treatment of adults and children with bacterial conjunctivitis.This was a multicenter, prospective, randomized, double-masked, vehicle-controlled, parallel-group study. Patients aged ≥1 year with bacterial conjunctivitis were randomized to receive besifloxacin ophthalmic suspension or vehicle administered twice daily for 3 days. There were 3 study visits: the baseline visit, visit 2 (day 4 or 5), and visit 3 (day 7±1). Participants recorded the times of medication instillation in a patient diary.

Tolerability assessments included ocular adverse events (AEs), changes in visual acuity, biomicroscopy and ophthalmoscopy findings, and nonocular AEs. Results for the individual clinical outcomes and microbial and clinical outcomes by gram-positive and gram-negative species were consistent with the primary efficacy outcomes.

In these adults and children with bacterial conjunctivitis, treatment with besifloxacin ophthalmic suspension 0.6% administered twice daily for 3 days was effective and well tolerated.

Wang Z *et al.*, (2012) worked on the enantiometry of Besifloxacin hydrochloride, a novel chiral broad-spectrum fluoroquinolone developed for the treatment of bacterial conjunctivitis and reported. R-besifloxacin hydrochloride is used in clinics as a consequence of its higher

antibacterial activity. To establish an enantiomeric impurity determination method, some chiral stationary phases (CSPs) were screened. Besifloxacin enantiomers can be separated to a certain extent on Chiral CD-Ph (Shiseido Co., Ltd., Japan), Chiral AGP, and Crownpak CR (+) (Daicel Chemical IND., Ltd., Japan). However, the selectivity and sensitivity were both unsatisfactory on these three CSPs. Therefore, Chiral AGP, Chiral CD-Ph, and Crownpak CR (+) were not used in the enantiomeric impurity determination of besifloxacin hydrochloride. The separation of enantiomers of besifloxacin was further performed using a precolumn derivatization chiral high-performance liquid chromatography method. 2,3,4,6-Tetra-O-acetyl-beta-D-glucopyranosyl isothiocyanate was used as the derivatization reagent. Besifloxacin enantiomer derivates were well separated on a C(18) column (250 × 4.6 mm, 5 μm) with a mobile phase that consisted of methanol-KH(2)PO(4) buffer solution (20 mm; pH 3.0) (50:50, v/v). Selectivity, sensitivity, linearity, accuracy, precision, stability, and robustness of this method were all satisfied with the method validation requirement. The method was suitable for the quality control of enantiomeric impurity in besifloxacin hydrochloride.

Malhotra R*et al.*,(2013) carried out a multi-centre randomized work in which the objective was to compare the safety of besifloxacin ophthalmic suspension 0.6 %, administered three times a day for 7 days, with that of its vehicle. This randomized, multicenter, double-masked, vehicle-controlled, parallel-group study involved 518 patients ≥1 year of age with a clinical diagnosis of bacterial conjunctivitis. Patients were randomized 2:1 to treatment with besifloxacin 0.6 % ophthalmic suspension or vehicle, one drop in the infected eye(s) TID for 7 days.

Thirty-one ocular treatment-emergent adverse events (TEAEs) were reported by 28 subjects in the study eye; 19 occurred in 17/344 (4.9 %) besifloxacin patients, and 12 occurred in 11/170 (6.5 %) vehicle patients (p = 0.5362). Only two ocular events (mild instillation site reaction, one case in each group) were considered "definitely related" to study treatment. One event of self-limited dysgeusia in the besifloxacin group was considered definitely related to treatment; there were no other nonocular TEAEs considered related to treatment.

These findings indicate that besifloxacin ophthalmic suspension 0.6 % is safe in patients aged 1 year and older when used TID for 7 days.

The study to evaluate the role of besifloxacin in bacterial conjunctivitis treatment was carried out by **Tracy D Mahvan *et al.*. (2014).** They concluded that Besifloxacin 0.6% ophthalmic suspension 3 times a day for 5 days is safe and effective for BC. Twice-a-day dosing for 3 days was also effective-a simplified regimen compared with other fluoroquinolones. Disadvantages include price and lack of a generic. Further evaluation is needed to evaluate comparative efficacy among other ocular fluoroquinolones and unlabeled uses.

Intracellular Targets of Fluoroquinolones:

Fluoroquinolones work by targeting and inhibiting DNA gyrase and/or Topoisomerase IV (Topo IV), both of which are type II bacterial topoisomerases involved in the DNA replication process. Type II topoisomerases function by breaking both strands of DNA, passing another segment of DNA through the break, and then religating the broken DNA. Specifically, they relax positive supercoils so that the DNA can unwind and replicate. More specifically, Topo IV's primary role is that of a decatenase, while gyrase's primary function is to relax positive supercoils. While DNA gyrase and Topo IV are similar in function, they are structurally distinct enzymes. Generally, DNA gyrase, composed of GyrA and GyrB subunits, is the primary target of Fluoroquinolones in Gram-negative bacteria, while Topo IV, composed of ParC and ParE subunits that are sometimes referred to as GrlA (names as gyrase-like) and GrlB subunits, is the primary target in Gram-positive bacteria. The ability of the fluoroquinolone class antibiotics to target type II topoisomerases offers several advantages over other antibiotic targets. First, type II topoisomerases are involved in DNA replication, an essential cell process that is universal to all bacteria. Second, type II topoisomerases are structurally unique enough from analogous human enzymes to allow for selectivity in targeting bacterial cells over human cells. Thirdly, the effect of target inhibition is bactericidal. Finally, Fluoroquinolones have the ability to potentially inhibit two distinct bacterial enzymes, thus reducing the possibility of selecting resistant mutants. All of these advantages demonstrate how fluoroquinolone class antibiotic agents have been, and will continue to be, attractive therapeutic options.

Mechanism of Fluoroquinolone Action:

The quinolone binds the DNA topoisomerase complex to form a ternary complex, which may lead to cell death by multiple pathways. Rapid cell death can occur via two different

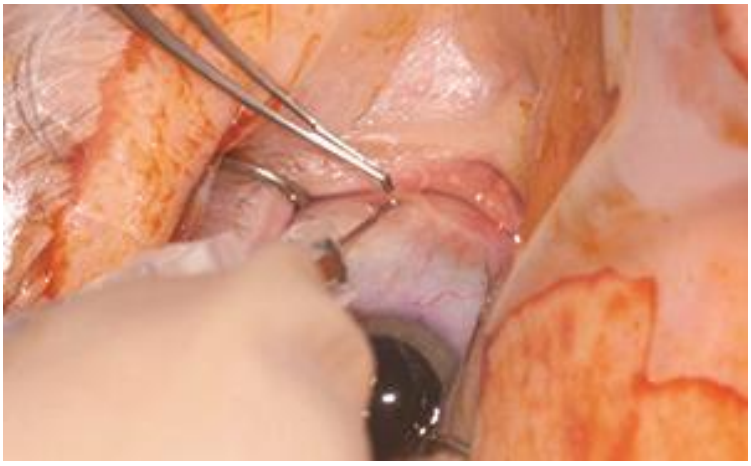
pathways: one requiring protein synthesis and one that does not. Cm = chloramphenicol, a known protein synthesis inhibitor. Adapted from Cm (protein synthesis-dependent) (proteinsynthesis-independent) SDS-dependent release of DNA breaks after cell lysis Blocks growth and form a ternary complex with the DNA and DNA gyrase/Topo IV, which then becomes trapped, disrupts replication, and triggers various cell death mechanisms. Topoisomerase binds and nicks each DNA strand in the replication process, with four DNA base pairs in between the nicked sites. The fluoroquinolone then binds the topoisomerase/nicked DNA complex in each of the nicked sites.

However, it is not certain as to what the exact order of binding is. Once the ternary complex is formed, different mechanisms of cell death are observed by different Fluoroquinolones. With older generation Fluoroquinolones, such as nalidixic acid and ciprofloxacin, slow cell death is observed. In vitro work with purified enzyme shows that ternary complex formation is fully reversible after the older generation drugs are removed because DNA religation is observed. However, with newer Fluoroquinolones, such as moxifloxacin, rapid cell death is observed. With these agents in the in vitro purified enzyme assays, all of the DNA is not religated after the fluoroquinolone is removed. In cells, this effect is observed as chromosomal fragmentation. Rapid cell death also appears to be able to occur via two different mechanisms: one requiring protein synthesis and the other in the absence of protein synthesis. For example, prior to MIC assays, chloramphenicol (Cm), a known protein synthesis inhibitor, can be added to stop protein synthesis. Some Fluoroquinolones will kill in the presence of Cm, while others do not. The structural requirements for a fluoroquinolone to kill or not kill in the presence of Cm (absence of protein synthesis) are not known. Additionally, fluoroquinolone interactions with DNA may contribute to which mechanism of cell death are observed.

The retrospective study included data from over 16,000 procedures and compared the rates of endophthalmitis over three different time periods during which endophthalmitis prophylaxis protocols varied. During 2007, only topical drops were used postoperatively. Intracameral cefuroxime was added except in patients with allergy or posterior capsule rupture during 2008 and 2009, while during 2010 and 2011, patients received both topical and intracameral antibiotics. However, the intracameral agent could be cefuroxime, moxifloxacin, or vancomycin based on surgeon preference, and there were no exclusions to cefuroxime use.

Rates of postoperative endophthalmitis declined from 0.31% with topical antibiotic drop use to 0.14% with initiation of intracameral antibiotics, and then to just 0.01% during the last 2 years of the study.

Fig. 10



Subconjunctival injection of a steroidal drug maintains the drug in the eye for as long as six weeks, eliminating another postop drop

But as much as the prophylactic approach in phacoemulcification of cataract cases is topical application of antibiotics of the surgeon's choice. Based on review of the available evidence and its quality, the strongest support exists for using povidone-iodine antisepsis and topically applied antibiotics **(Packer M, *et al.*, 2012).** Moxifloxacin 0.5% ophthalmic solution (Vigamox, Alcon) is being used by some surgeons because it is a convenient, off-the-shelf, preservative-free formulation, and it appears to have good safety in terms of risks of toxic anterior shock syndrome (TASS) and CME according to several published reports. The current trend in busy ophthalmic centres is the fourth generation fluoroquinolone antibiotic - Besifloxacin that has almost the same three advantageous points similar to moxifloxacin. For those three reasons—tonicity, pH and absence of preservative—off-the-shelf Besivance® is compatible with the intraocular environment and is tolerated intracamerally too without dilution."

Hence **Besifloxacin** has been taken up for this study to investigate penetration and effectiveness on topical application through conventional mode of administration and in pulse mode of administration.

Drug Profile

III. DRUG PROFILE

BESIFLOXACIN

CLASS : Ophthalmological Anti-infective

DESCRIPTION : **Besifloxacin** is a fourth-generation fluoroquinolone antibiotic.

Ophthalmic suspension used to treat bacterial conjunctivitis.

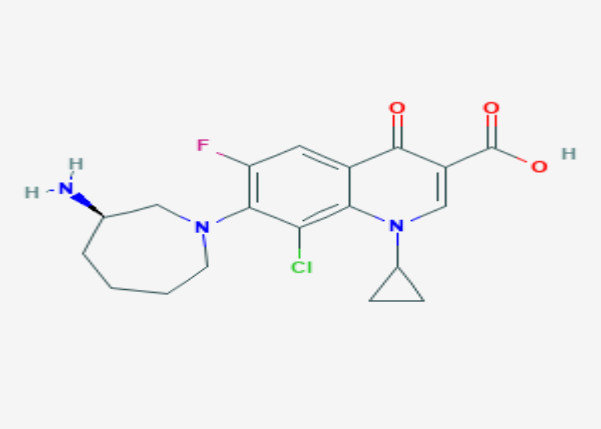
COMMON BRAND(S): Besipact; Besivance

GENERIC NAME(S): BesifloxacinHCl

MOLECULAR WEIGHT: 393.843 g/mol

MOLECULAR FORMULA: C19H21CLFN3O3

MOLECULAR STRUCTURE:



MECHANISM OF ACTION:

Besifloxacin is a bactericidal fluoroquinolone–type antibiotic that inhibits bacterial enzymes, DNA gyrase and topoisomerase IV. By inhibiting DNA gyrase, DNA replication, transcription, and repair is impaired. By inhibiting topoisomerase IV, decatenation during cell division is impaired. Inhibiting these two targets also slows down development of resistance.

DOSAGE &INDICATION

Treatment of bacterial conjunctivitis

Bacterial isolates that are susceptible to besifloxacin include:

- *Corynebacteriumpseudodiphtheriticum*
- *Corynebacterium striatum*
- *Haemophilus influenza*
- *Moraxella lacunata*
- *Staphylococcus aureus*
- *Staphylococcus epidermidis*
- *Staphylococcus hominis*
- *Staphylococcus lugdunensis*
- *Streptococcus mitis*
- *Streptococcus ralis*
- *Streptococcus pneumonia*
- *Streptococcus salivarius*

OPHTHALMIC DOSAGE

Adults, Adolescents, and Children

Instill 1 drop in the affected eye(s) 3 times daily, 4 to 12 hours apart, for 7 days.

CONTRAINDICATION / PRECAUTIONS

General Information

Besifloxacin is for topical ophthalmic use only and should not be injected subconjunctivally or introduced directly into the anterior chamber of the eye.

Quinolone hypersensitivity

The manufacturer does not specifically provide a caution in using besifloxacin, an 8-chloro fluoroquinolone, in patients with a history of quinolone hypersensitivity; however, it seems reasonable that caution should be exercised in cases of known allergy.

Contact lenses

Patients should not wear contact lenses during the treatment of bacterial conjunctivitis with besifloxacin.

Fungal infection

Prolonged use of besifloxacin may result in the overgrowth of non-susceptible organisms. If superinfection, including fungal infection, occurs, patients should discontinue besifloxacin use and institute alternative therapy.

Geriatric

No safety and efficacy differences with besifloxacin have been reported between geriatric and younger patients.

Infants, neonates

The safety and efficacy of besifloxacin have not been established in neonates and infants less than 1 year of age. Efficacy of besifloxacin in children one year and older has been established in clinical trials. The manufacturer states that there is no evidence that ophthalmic administration of quinolones has any effect on weight bearing joints.

Pregnancy

No adequate and well-controlled studies with besifloxacin have been conducted in pregnant women and its ability to cause fetal harm or affect reproductive capacity is unknown. In animal studies, the No Observed Adverse Effect Level (NOAEL) for embryo-fetal development was 100 mg/kg/day (Cmax 5 mcg/mL or greater than 11,000-times the mean serum concentration measured in humans).

Low serum concentrations have been observed during ophthalmic administration (e.g., less than 1.3 ng/mL). To minimize the amount of drug that reaches systemic circulation, apply pressure over the tear duct by the corner of the eye for 1 minute after each administration. The manufacturer recommends that besifloxacin be used during pregnancy only if the potential benefits to the mother outweigh the potential risk to the fetus.

Breast-feeding

There are no data on the presence of besifloxacin in human milk, the effects on the breastfed infant, or the effects on milk production. It is not known whether measurable concentrations of besifloxacin would be present in maternal milk after topical ocular administration; however, systemic exposure after ocular administration is low. The low maternal serum concentrations (less than 1.3 ng/mL) suggest exposure to a nursing infant would likely be clinically insignificant. To minimize the amount of drug that reaches systemic circulation and breast milk, apply pressure over the tear duct by the corner of the eye for 1 minute after each administration. The manufacturer suggests caution when administering to lactating women. Consider the benefits of breast-feeding, the risk of potential infant drug exposure, and the risk of an untreated or inadequately treated condition. If a breast-fed infant experiences an adverse effect related to a maternally administered drug, health care providers are encouraged to report the adverse effect to the FDA.

PHARMACOKINETICS

Besifloxacin is applied topically to the eye. Systemic absorption is minimal. The mean elimination half-life after multiple doses was estimated to be 7 hours.

Ophthalmic Route

Systemic absorption of besifloxacin is minimal. Serum concentrations were measured in adults with suspected bacterial conjunctivitis receiving besifloxacin bilaterally three times daily (16 doses total). After both the first and last dose, maximum serum concentrations were less than 1.3 ng/mL with a mean Cmax of 0.37 ng/mL on day 1 and 0.43 ng/mL on day 6.

MAXIMUM DOSAGE

Adults

3 drops/eye/day.

Elderly

3 drops/eye/day.

Adolescents

3 drops/eye/day.

Children

3 drops/eye/day.

Infants

Safety and efficacy have not been established.

DOSING CONSIDERATIONS

Hepatic impairment

Specific guidelines for dosage adjustment in hepatic impairment are not available; it appears that no dosage adjustments are needed.

Renal Impairment

Specific guidelines for dosage adjustment in renal impairment are not available; it appears that no dosage adjustments are needed.

ABSORPTION:

Although ocular surface concenetrations are high, average systemic concenetrations after three –times daily dosing was less than 0.5 ng/mL. This indicates that besifloxacin is not appreciably absorbed into the systemic and has a very low risk of systemic side effects.

Volume of distribution:

Not absorbed into the systemic

Protein binding:

None

Metabolism :

No appreciable metabolism

Route of elimination:

N/A

Half life:

The average elimination half –life of besifloxacin in plasma following multiple dosing was estimated to be 7 hours.

Clearance:

N/A

Toxicity:

LD50 Rat: >2000mg/kg. The most common adverse effects reported in 2% of patients treated with besifloxacin was conjunctival redness.

PHARMACODYNAMICS

Besifloxacin is a fluoroquinolone that has a broad spectrum in vitro activity against a wide range of Gram-positive and Gram-negative ocular pathogens: ***e.g.,***

- *Corynebacterium pseudodiphtheriticum*
- *Moraxella lacunata*
- *Staphylococcus aureus*
- *Staphylococcus epidermidis*
- *Staphylococcus hominis*
- *Streptococcus mitis*
- *Streptococcus oralis*
- *Streptococcus pneumoniae and*
- *Streptococcus salivarius.*

Besifloxacin has been found to inhibit production of pro inflammatory cytokines *in vitro*. The mechanism of action of besifloxacin involves inhibition of two enzymes which are essential for the synthesis and replication of bacterial DNA: the bacterial DNA gyrase and topoisomerase IV.

DRUG INTERACTION/S

There are no drug interactions associated with Besifloxacin products.

ADVERSE EFFECTS

During the treatment, the most frequently reported ocular adverse reaction was the appearance of conjunctival redness (approximately 2% of patients). Other possible adverse reactions, reported in subjects treated with besifloxacin were: eye pain, itching of the eye, blurred vision, swelling of the eye or eyelid, severe dizziness, troubled breathing.

Moderate ADR

Blurred vision / Early / 1.0-2.0

Conjunctivalhyperemia / Early / 2.0-2.0

Superinfection / Delayed / Incidence not known

Mild ADR

Ocular irritation / Rapid / 1.0-2.0

Ocular pruritus / Rapid / 1.0-2.0

Ocular pain / Early / 1.0-2.0

Headache / Early / 1.0-2.0

OPHTHALMIC ADMINISTRATION

- For topical ophthalmic administration only
- Wash hands prior to use
- Remove contact lenses prior to use
- Prior to administration, invert the closed bottle and shake once
- Remove the cap while the bottle is in the inverted position and administer one drop into the affected eye(s)
- Do not rinse the dropper after use; keep container tightly closed
- Use one bottle per patient; do not share ophthalmic drops

STORAGE & DISPOSAL OF BESIFLOXACIN

- Store at room temperature. Do not freeze.
- Protect from light.
- Keep all drugs in a safe place. Keep all drugs out of the reach of children and pets.
- Check with your pharmacist about how to throw out unused drugs.

Need for the Present Study

IV. NEED FOR THE PRESENT STUDY

According to World Health Organization, Cataract is the preventable blindness throughout the world. Unlike Infectious disease Specialists of Internal Medicine, Ophthalmologists have the advantage of being able to place an antibiotic agent directly on the target tissue (topically) without excessive direct concern for issues such as volume of distribution or the metabolism of the drug. Whether the prophylactic effect of topical administration of antibiotics in cataract surgery can be augmented by the addition of *intracameral* administration remains a topic of discussion.

Ocular Pharmacokinetics of the Anterior Segment

The anterior segment of the eye constitutes

- CORNEA
- CONJUNCTIVA
- AQUEOUS HUMOR
- LENS
- IRIS AND CILIARY BODY (ICB).

The primary routes for drug delivery to the anterior segment include -

- Topical administration
- Subconjunctival, and
- Intracameral injections.

Pharmacokinetics

Pharmacokinetic processes involved with each of these major routes of administration are as follows:

After Topical Administration:

Topical administration is the most convenient route of drug delivery to the anterior segment of eye. Following topical instillation, majority of the administered drug is cleared rapidly from the ocular surface resulting in only 1–7% of the dose to reach the aqueous humor **(Ghate and Edelha user 2006)**. Precorneal clearance mechanisms including tear fluid

turnover and blinking, selective permeability of the corneal epithelial barrier, and drug loss through naso-lacrimal as well as systemic circulation attribute to the low bioavailability of drugs administered by this route.

Factors Affecting Absorption and Bioavailability

The critical factors that may affect the absorption process and alter the intraocular bioavailability of topical drops consist of physiological factors relevant to ocular tissues and molecular properties unique to drugs. A complete understanding of the interaction between these factors is essential to enhance the pharmacokinetic processes.

Loss of Drug from the Precorneal Surface

The tear volume in humans under normal condition is 7–9 μL with a turnover rate of 0.5–2.2 μL/min. Many commercially available eyedroppers deliver a typical volume of 25–56 μL to the precorneal tear film resulting in an increase in the tear volume. Under normal conditions, human palpebral fissure can hold 30 μL without overflowing. This abrupt increase in the volume due to topical instillation causes reflex blinking and rapid drainage from ocular surface. Majority of the applied medication is drained from the surface through the nasolacrimal duct and eventually cleared via systemic circulation.

Drug Properties Affecting Absorption

In case of topically administered drugs, absorption through cornea may occur via transcellular or paracellular pathways or by active transport. Drug properties that influence these processes such as lipophilicity and aqueous solubility play a key role in the penetration of drugs across cornea. Lipophilicity (LogP) in the range of 2–3 was found to be optimal for corneal permeation of steroids and β-blockers **(Schoenwald and Huang 1983).** An exploratory analysis of the apparent corneal permeability (Papp) values for more than 100 compounds indicated that corneal permeability is dependent on the distribution coefficient - LogD at experimental pH **(Prausnitz and Noonan 1998)**. As the dataset was mostly comprised of small molecules, no apparent dependency on molecular weight was observed. Further analysis of this permeability data with other molecular descriptors revealed potential correlation of corneal permeability with polar surface area (PSA).

PSA is the sum of surfaces of polar atoms, primarily oxygen, nitrogen, and their attached hydrogen atoms. PSA along with lipophilicity and molecular size influence the passive diffusion of molecules. Due to the hydrophilic nature of corneal stroma, highly lipophilic compounds have limited permeability across this tissue. Stromal permeability data from a limited number of molecules (N ¼ 19) indicated a strong dependence on its molecular weight and radius but no apparent relationship with any of the lipophilicity indicators (LogP or LogD) **(Prausnitz and Noonan 1998)** . As indicated earlier, stroma is a thick, fibrous, and hydrophilic tissue where diffusion plays a major role in the transport of molecules. Thus stromal permeability is negatively correlated with molecular weight and radius, the parameters that affect the diffusion of a compound. Permeability of corneal endothelium shows a good correlation with both LogD and molecular radius indicating the role of both lipophilic and hydrophilic pathways. Similar to intact cornea, an increase in the corneal endothelial permeability was observed with a moderate increase in lipophilicity. However, the data was limited by the absence of highly lipophilic compounds to further investigate the barrier properties. Due to the presence of large intercellular junctions and leaky nature of the endothelial layer, strong correlation was observed with molecular radius. In general, taking into consideration the overall data, corneal epithelium serves as main barrier to transport of molecules across cornea. Small molecules with favourable lipophilicity readily cross corneal epithelium but stroma may provide a barrier to macromolecules. Several formulation approaches are employed to overcome the absorption barriers and improve the ocular bioavailability. More information on these can be found in a recent review article **(Ghate and Edelhauser 2006).**

Distribution of Drugs in the Anterior Segment of Eye:

Topically administered drugs permeate across the cornea and enter the aqueous humor followed by distribution to the surrounding ocular tissues including iris–ciliary body, lens, choroid–retina, and vitreous **(Ghate and Edelhauser 2006)**. Drugs that may exhibit non-corneal routes of absorption enter the uveal tract and vitreous without entering the aqueous humor. The rate and extent of drug distribution in the anterior segment is determined by a number of factors including permeability, diffusion in the aqueous humor, binding to proteins and surrounding ocular tissue components. Most of these factors are influenced by a drug's physicochemical properties including lipophilicity, solubility, and molecular weight.

The apparent volume of distribution (Vd) of drugs can be measured by direct administration into the aqueous humor (intracameral). However, there is a paucity of data on the pharmacokinetics of drugs following intracameral injection. Based on the volume of aqueous humor in rabbits (0.3 mL), the Vd ranged from two- to tenfold larger than the aqueous humor volume. Although no clear trend was observed between Vd and molecular weight or LogP, drugs with higher protein binding had a lower Vd in the aqueous humor. Drugs that extensively bind to plasma proteins were known to exhibit a low Vd and can have a long plasma half-life. Flurbiprofen, a highly protein bound drug, has a longer elimination half-life in aqueous humor when compared to other moderate to weakly bound drugs. For topically administered drugs, protein binding occurs first in the tear fluid which has a rapid turnaround time and as a result only the free unbound drug is available for corneal absorption. More binding of the absorbed drug occurs in the cornea and aqueous humor. Protein content of the aqueous humor is different when compared to plasma. Concentration of proteins in the aqueous humor is approximately 200 times less than in plasma. However, these levels may increase in certain disease states that involve inflammatory conditions and subsequently result in increased binding of drugs. The effect of protein binding was investigated by adding increasing amounts of rabbit serum albumin to pilocarpine solution before topical administration **(Mikkelson*et al.*, 1973).** The results indicated a 75- to 100-fold reduction in response (pupillary diameter) by the addition of 3% albumin indicating a decreased bioavailability as a result of protein binding. Nevertheless, more data comparing the pharmacokinetics of drugs in normal versus diseased state (e.g., inflammation, blood-aqueous barrier breakdown, etc.) is required to understand the effect of protein binding on the disposition of topically administered drugs. From a therapeutic perspective, distribution of a drug to its target site is essential to achieve the desired efficacy. Although measurement of drug concentration in the aqueous humor provides an estimation of Vd, measuring drug levels in the surrounding ocular tissues is required to assess if the drug has reached the site of action. While pharmacokinetic studies with extensive tissue distribution data are scarce, few studies report the drug concentrations in key ocular tissues in addition to the aqueous humor. Given the number of animals required and the destructive nature of tissue sample collection, this is not uncommon in the ophthalmology field.

As expected, the relative exposure was higher in the cornea following topical instillation of drugs. The relative exposure in cornea was several folds higher for high molecular weight compounds (azithromycin and cyclosporin) when compared to other drugs. Also, the relative

exposure of drugs in iris–ciliary body is higher than in aqueous humor. With the exception of lomefloxacin, for which data was available from infected rabbit eyes, the relative exposure in ICB decreased with increasing lipophilicity (LogP). Several explanations have been postulated to explain this higher exposure in ICB **(Schoenwald 2003).** Iris of rabbit eye is a porous and highly vascular tissue with majority of its surface area exposed to aqueous humor thereby allowing extensive distribution from aqueous humor. Further, an increased affinity/capacity for binding to melanin pigment in the iris could enhance the distribution to this tissue. Brimonidine, a drug well known to bind melanin, has higher relative exposure in ICB than in cornea. Levobetaxolol, a cardioselective beta-adrenergic receptor blocking agent, has higher affinity to melanin with ICB exposure several folds higher than in aqueous humor.

An alternative explanation for the higher exposure in ICB could be due to potential contribution of non-corneal absorption routes via conjunctival/scleral pathways. Based on their physicochemical properties, certain drugs may be preferentially absorbed by conjunctiva and sclera to reach ICB without entering the aqueous humor. **Chien DS.*et al.*, (1990)** investigated the ocular absorption via corneal and conjunctival/scleral routes of clonidine, p-aminoclonidine, and AGN 190342 after drug perfusion in vivo. When drug was maintained over the conjunctiva over a period of time, the rank order of drug concentration in the anterior chamber tissues was:

Conjunctiva >Cornea >Ciliary body >Aqueous humor;

whereas, when drug solution was in contact with cornea, the rank order for tissues was:

Cornea >Aqueous humor>Ciliary body >Conjunctiva.

Besides, the conjunctival/scleral pathway was contributed as the predominant pathway for the least lipophilic (p-aminoclonidine) compound. Further experiments carried out using beta-blocking agents with varying lipophilicity, sucrose and inulin demonstrated that the outer layer of sclera provides less resistance to penetration of hydrophilic drugs when compared to cornea **(Ahmed and Patton 1985).** Moreover, the estimated permeability of conjunctival and scleral tissues was found to be 15–25 times higher than the cornea and was not affected by molecular size **(Hamalainen *et al.*, 1997).**

In more recent data from the Bascom Palmer Eye Institute between 2000 and 2005, the rate of endophthalmitis after cataract surgery did not increase significantly with topically applied fluoroquinolones **(Miller *et al.*, 2006).** Risk factors for endophthalmitis between 2000 and 2005, included systemic immuno-suppression, the method of operative preparation, intra-operative complications such as vitreous loss, peri operative factors such as the presence of surface bacteria, wound construction factors such as wound leak and inferior incision placement, and the presence of chronic blepharitis.

The role of topical antibiotic prophylaxis is to reduce the colonization with the organisms of the periocular flora on the ocular surface, as the patient's own flora are often the cause of endophthalmitis. Other studies support that gram-positive, coagulase-negative organisms, notably *Staphylococcus epidermidis*, are the predominant causative agents in cases of endophthalmitis **(Han *et al.*,1996** and**Speaker MG & Menikoff JA. 1991).**

Goals of prophylaxis

The goals of prophylaxis must be taken into account when considering a Regimen to reduce the risk for infection.

A second goal of antibiotic prophylaxis is to eradicate the organisms that may enter the eye. Natural host defence mechanisms in the eye are efficient at removing inoculations to a degree, but a therapeutic concentration of antibiotic is needed when the contamination exceeds the capacity of the aqueous humour to adequately clear an organism. Possible modes of administration include *sub-conjunctival* injections, placement of antibiotics in the infusion fluid, oral systemic administration and topical administration.

Although a recent study by members of the European Society of Cataract and Refractive Surgeons concluded that *intracameral* administration of cefuroxime reduced the incidence of postoperative endophthalmitis, the preparation used for *intracameral* administration in the study is neither commercially available in the United States nor approved for ophthalmic use by the US FDA **(Barry *et al.*, 2006).** Because of the need for compounding antibiotics, errors in dilution or loss of sterility may occur. Rarely, patients exhibit hypersensitivity

to beta lactam antibiotics. In addition, this cephalosporin is not effective against methicillin-resistant *Staphylococcus aureus* (MRSA) or methicillin-resistant *Staphylococcus epidermidis* (MRSE), *Enterococcus faecalis* or *Pseudomonas aeruginosa*. With multiple doses prepared in a single container, contamination with *P aeruginosa* may occur.

With a compounded antibiotic preparation administered intraocularly, the possibility of toxic anterior segment syndrome (TASS) exists. TASS is an acute, noninfectious sterile inflammatory condition of the anterior segment that results from a number of causes, including irrigating solutions or medications outside a tolerable range of pH, medications in toxic concentrations, cleaning agents, organic material biofilms or toxic residues **(Mamalis N *et al.,* 2007).** An increased incidence of cystoid macular edema has also been reported in relation with use of *intracameral* antibiotics in the infusion fluid **(Axer-Siegel R *et al.,* 1999).**

Considering all the above facts of varying formulation parameters of antimicrobial eye drops for topical administration and the pharmacokinetic profile of these compounds the hitherto not much explored fluoroquinolone is taken up for this study.

The current trend in busy ophthalmic centres is the fourth generation fluoroquinolone antibiotics. A lot of clinical data is available on the fourth Gen Moxifloxacin and Gatifloxacin and on the third generation Levofloxacin. Whereas not much clinical data is available on Besifloxacin, yet another fourth gen fluoroquinolone that has almost the same three advantageous points similar to moxifloxacin.For those three reasons—tonicity, pH and absence of preservative—off-the-shelf Besivance® is compatible with the intraocular environment and is tolerated intracamerally too without dilution."

Hence **Besifloxacin** has been taken up for this study to investigate its corneal penetration power and its effectiveness on topical application by two modes namely the 'Conventional two-days mode' of administration and the 'Pulse mode' of administration an hour before the procedure.

Aim & Objective

V. AIM & OBJECTIVE

AIM

The aim is to study the bactericidal activity on conjunctival flora of topically applied *Besifloxacin* by two different modes of administration

- The Conventional mode of administering one drop of sterile antimicrobial eye drop since two days prior to surgery four times a day**(Regimen A)**
  &
- The Pulse modeof administration starting an hour immediately preceding surgery – 6 doses delivered every 10 minutes**(Regimen B)**

OBJECTIVE

- To determine, the penetration of *Besifloxacin* in aqueous humour withdrawn from the anterior chamber, by High Performance Liquid Chromatography

- To study the prevalence of associated diseases that cause cataract secondarily

- To find the type of cataract that prevails more in the study groups

- To find the most effective way of administering the drops for better penetration of the eyes

Plan of the Work

VI. PLAN OF THE WORK

The entire study has been planned to be carried out for a period of nine months from NOVEMBER 2017 to AUGUST 2018. The proposal was designed as given below:

NOVEMBER- DECEMBER 2017 (2 Months)

- Literature Review
- Obtaining consent from Hospital authorities
- Study Design and Data Entry Format (Proforma)
- Obtaining approval from Institutional Ethics Committee (IEC)

JANUARY- JUNE 2018 (6 Months)

- Selection of Patients
- Collection of Patients' details
- Collection of Samples
- Collection of Lab and other investigation Reports

JULY-AUGUST- SEPTEMBER 2018 (2 months)

- Compilation
- Data analysis
- Submission of Report

Methodology

VII. METHODOLOGY

MATERIALS AND METHODS

Study site : Vasan Eye Care Hospital, Tiruchirappalli

Study period: The study was conducted during a nine month period.

Inclusion criteria:

- The subjects of either sex (otherwise healthy) who were scheduled to undergo standard cataract surgery
- 21years of age or older
- Having a normal or lesser intraocular pressure
- Had the ability to understand and give signed informed consent.

Exclusion criteria:

- Those that exhibited any intraocular inflammation
- Had a known sensitivity to any of the ingredients in the study medications
- Had a known sensitivity to any quinolone compound
- Had a condition that may confound the study results or may interfere significantly with the subject's participation in the study
- Had a greater intraocular pressure
- Exhibiting a corneal ulcer, keratitis, or had a history of herpetic keratitis,
- Had any ocular disease that would interfere with the evaluation of the study treatments
- Received previous intraocular silicone oil
- Was pregnant or nursing.

METHOD OF STUDY

Fifty select patients undergoing cataract extraction were divided randomly into two groups with 0.5% Besifloxacin ophthalmic solution topically applied in both the groups:

- 0.5%Besifloxacin-25 patients under REGIMEN–A and
- 0.5%Besifloxacin-25 patients under REGIMEN-B.

Regimen A: 1 drop four times a day for two days (8 drops) – 25 patients and

Regimen B: 1 drop – 6 doses - delivered every 10 min in the hour immediately preceding surgery (6 drops) -25 patients.

Regimen A:

Two days before surgery the patient's conjunctival smear was taken as a baseline. Then the assigned drug was given randomly to each patient at a dose of a drop four times a day for two days. After two days of administration at the time of surgery a second smear was taken to assess the bacterial load to find the efficacy of the drug under Regimen A.

Regimen B:

Conjunctival smear was taken as a baseline before start of administration of Besifloxacin on the day of surgery and the drug administered every tenth minute a drop for an hour; and at the time of surgery a second smear was taken to assess the bacterial load to find the efficacy of the drug under Regimen B.

Fig. 11

Various Ophthalmic Routes of Administration

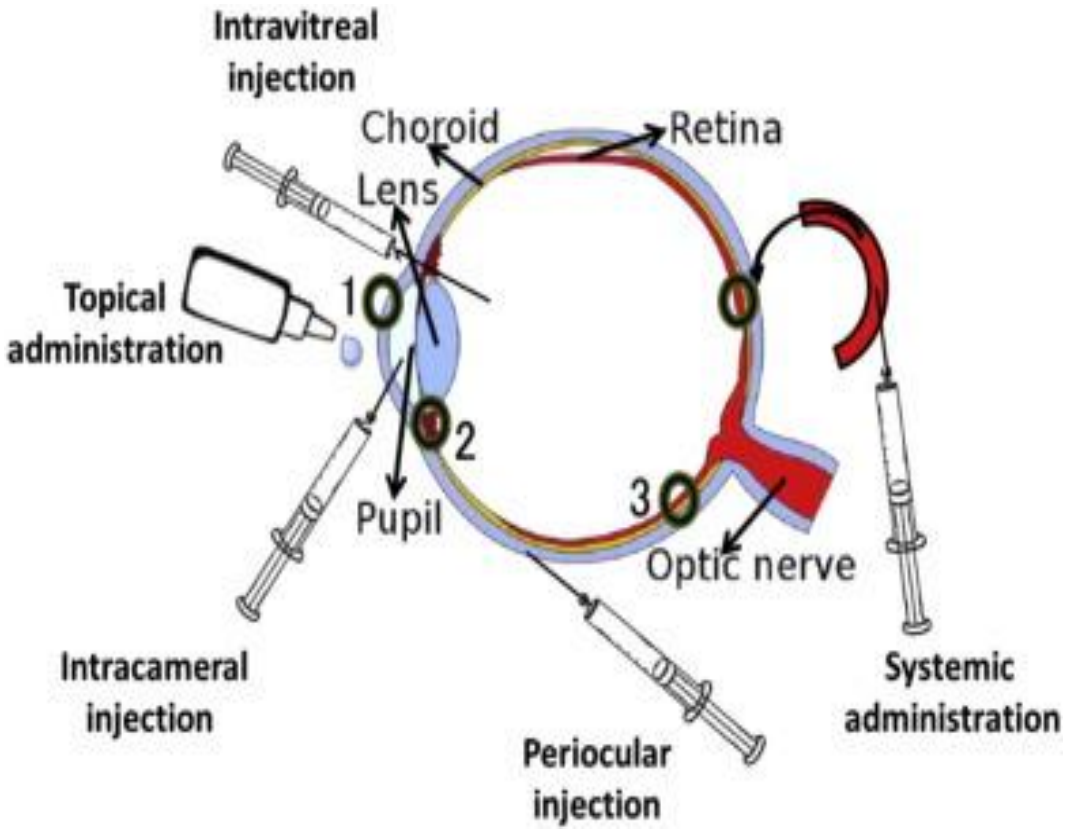


Fig. 12

Structures of the eye -labelled

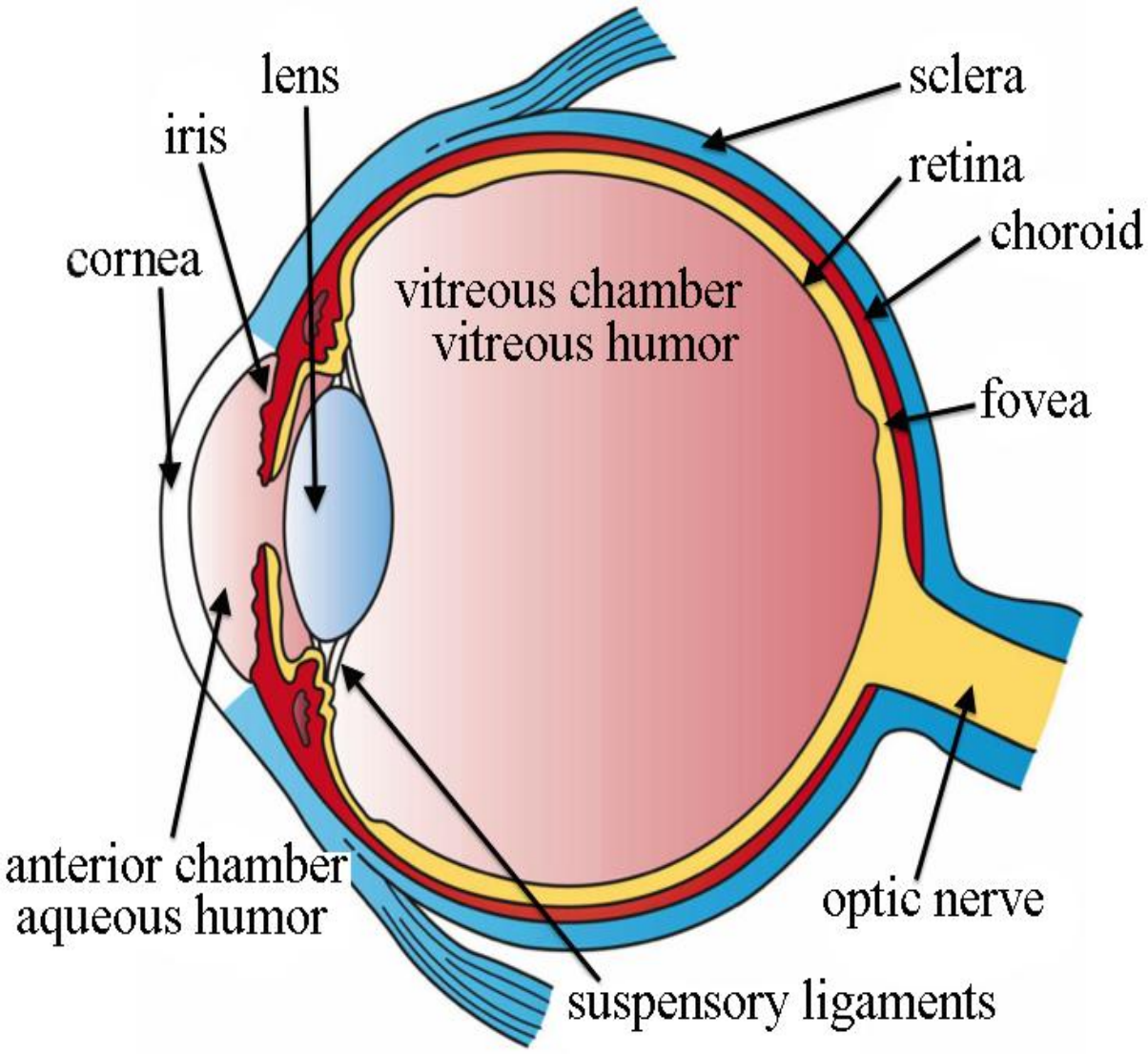
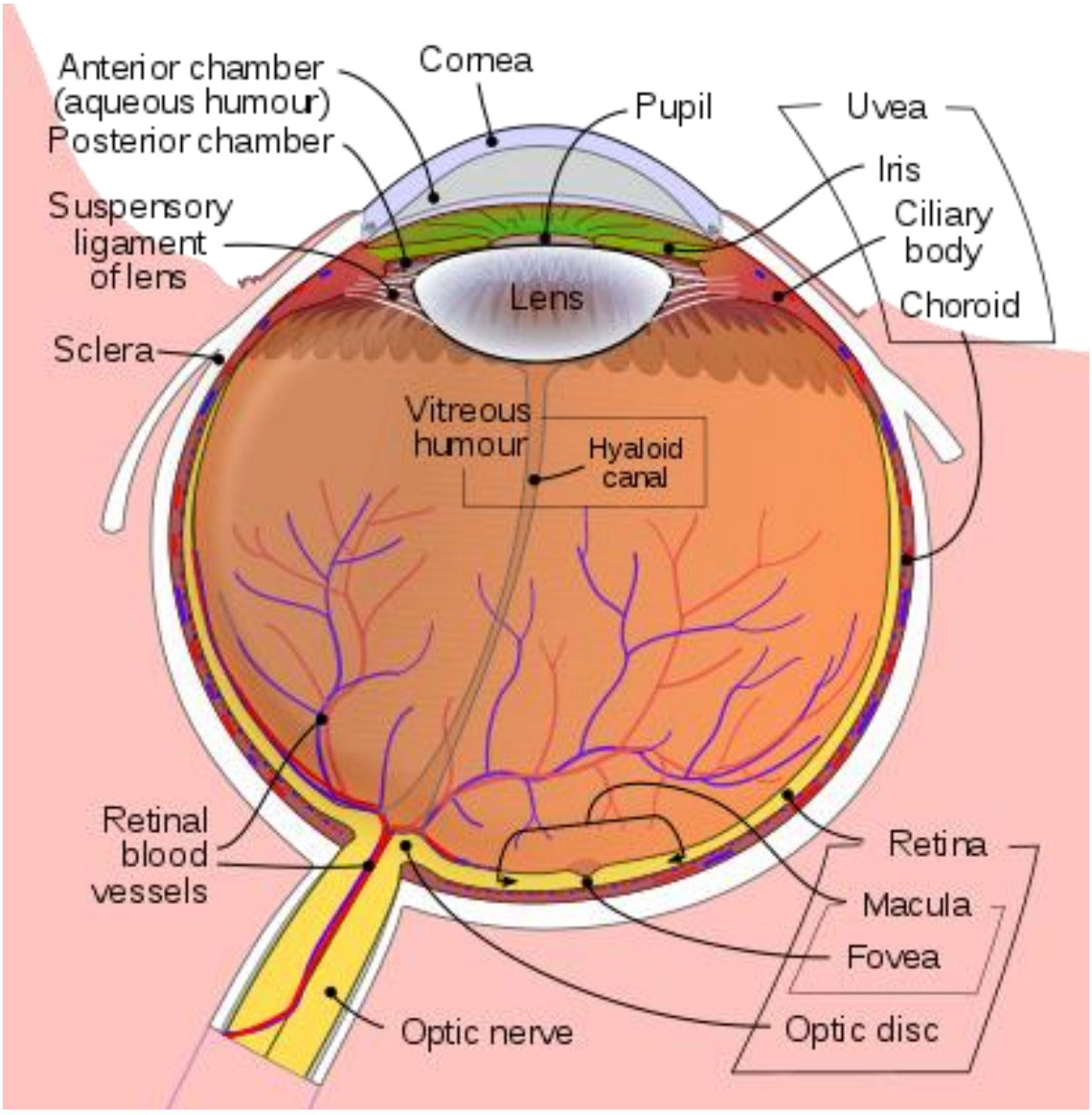


Fig. 13

The lower part of the right eye after a Central and Horizontal section



During surgery 0.2 ml of aqueous humour was aspirated with the help of a tuberculin syringe. The anterior chamber fluid was immediately placed into a sterilized cryogenic tube and stored at **-40° C** in an refrigerator and kept frozen until analysis was performed. Each tube was marked with a patient identification number (which indicated the antibiotic that the subject was randomly assigned), the subject's initials, date of surgery, and eye (R/L).

Conjunctival swab material was inoculated into 0.3 ml of sterile saline in a sterile vial. The swab was repeatedly twirled in the saline. In the laboratory, 0.1ml of the saline was inoculated onto a plate of 5% sheep blood agar while 0.05 ml each was inoculated onto a plate of **Nutrient agar** and a plate of **MacConkey agar**.

Nutrient Agar is a general purpose, nutrient medium used for the cultivation of microbes supporting growth of a wide range of non-fastidious organisms. Nutrient agar is popular because it can grow a variety of types of bacteria and fungi, and contains many nutrients needed for the bacterial growth.

TABLE: 1

Composition of Nutrient Agar

Ingredients	Note
0.5% Peptone (Pancreatic digest of gelatin)	*It is an enzymatic digest of animal protein. Peptone is the principal source of organic nitrogen for the growing bacteria*
0.3% beef extract/yeast extract	It is the water-soluble substances which aid in bacterial growth, such as vitamins, carbohydrates, organic nitrogen compounds and salts
1.5% agar	It is the solidifying agent
0.5% NaCl	*The presence of sodium chloride in nutrient agar maintains a salt concentration in the medium that is similar to the cytoplasm of the microbes*
Distilled Water	*Water is essential for the growth of and reproduction of micro-organisms and also provides the medium through which various nutrients can be transported.*

Final pH is adjusted to neutral (7.4) at 25 °C.

Preparation of Nutrient Agar

1. Suspend 28 g of nutrient agar powder in 1 litre of distilled water

2. Heat this mixture while stirring to fully dissolve all components

3. Autoclave the dissolved mixture at 121°C for 15 minutes

4. Once the nutrient agar has been autoclaved, allow it to cool but not solidify

5. Pour nutrient agar into each plate and leave plates on the sterile surface until the agar has solidified

6. Replace the lid of each Petri dish and store the plates in a refrigerator

Uses of Nutrients Agar

1. It is frequently used for isolation and purification of cultures

2. It can also be used as a means for producing the bacterial lawns needed for antibiotic sensitivity tests. In actuality, antibiotic sensitivity testing is typically performed on media specially formulated for that purpose

Fig. 14 *S. aureus* **in nutrient agar**

Fig. 15 *S. epidermidis* **in nutrient agar**

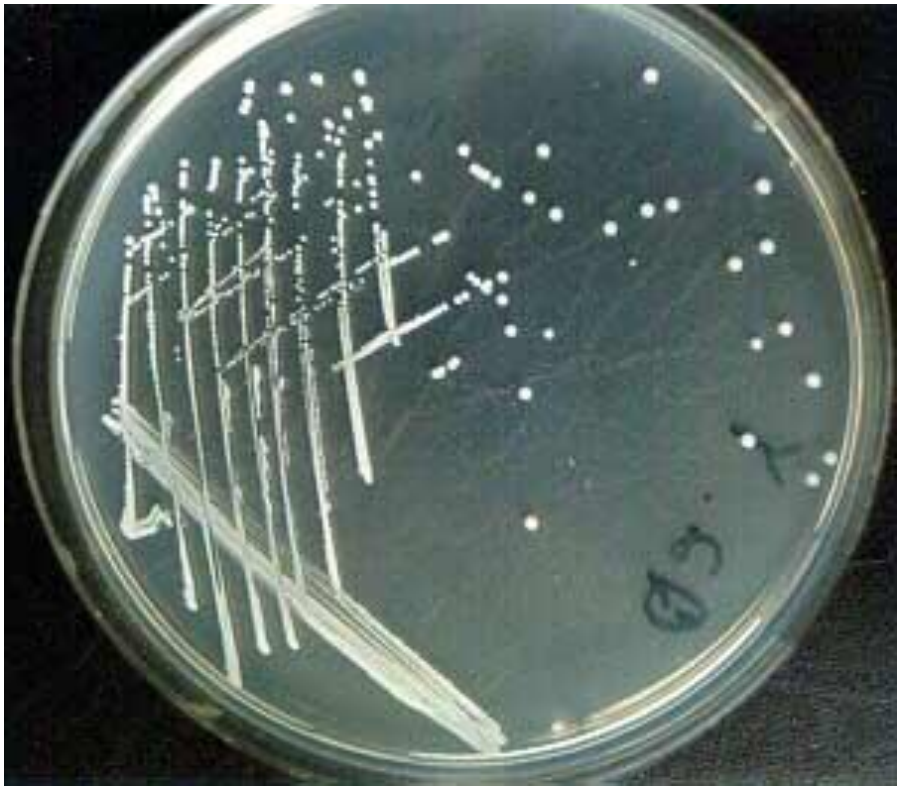
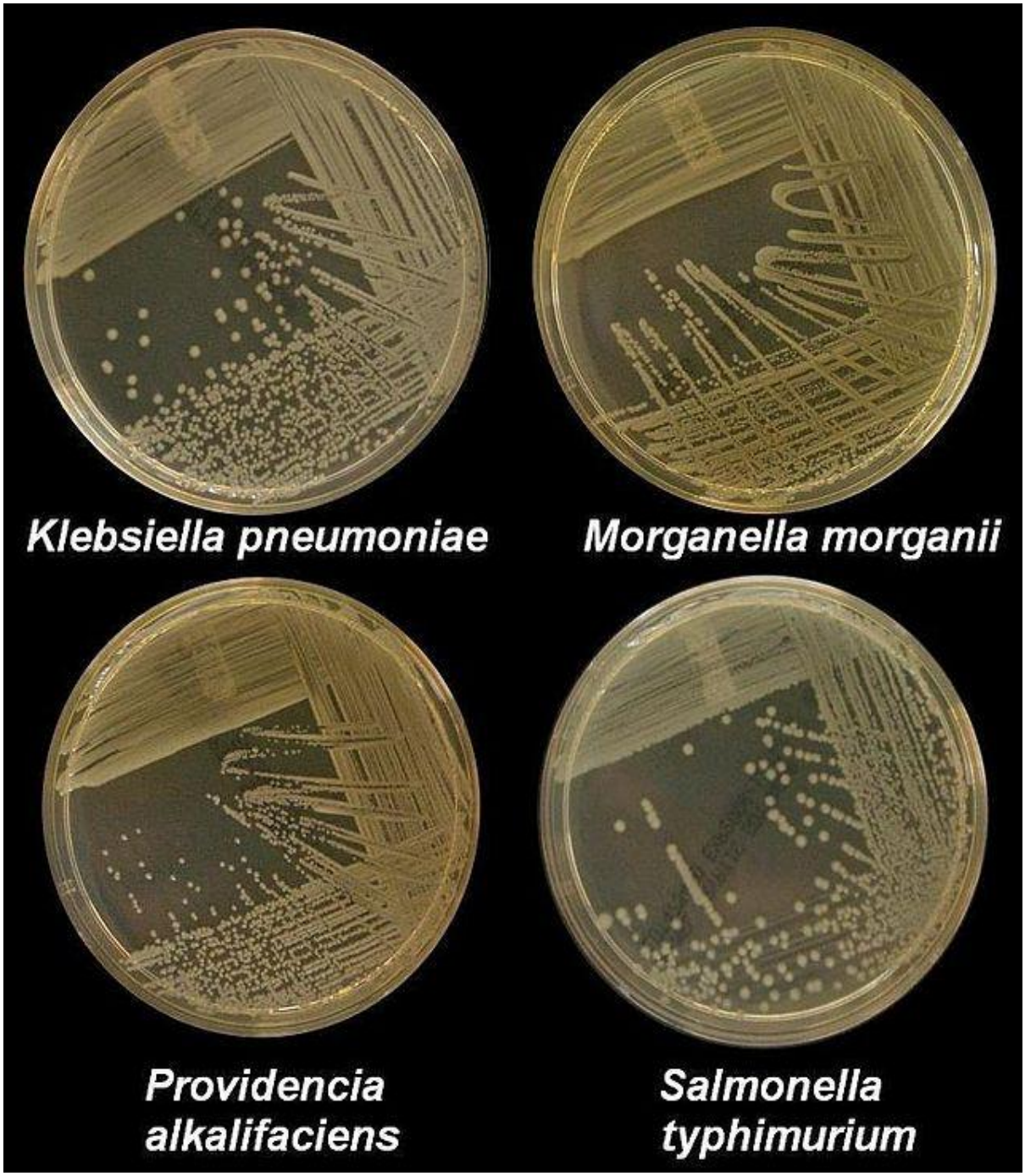


Fig. 16

Four nutrient agar plates showing colonies of common Gram negative *bacteriae*



MacConkey agar (MAC) is a selective and differential media used for the isolation and differentiation of non-fastidious gram-negative rods, particularly members of the family Enterobacteriaceae and the genus *Pseudomonas*.

TABLE: 2

Composition of MacConkey Agar

Ingredients	Amount
Peptone (Pancreatic digest of gelatin)	**17 gm**
Proteose peptone (meat and casein)	**3 gm**
Lactose monohydrate	**10 gm**
Bile salts	**1.5 gm**
Sodium chloride	**5 gm**
Neutral red	**0.03 gm**
Crystal Violet	**0.001 g**
Agar	**13.5 gm**
Distilled Water	**Add to make 1 Litre**

Final pH 7.1 +/- 0.2 at 25 degrees C.

Uses of MacConkey Agar

1. MacConkey agar is used for the isolation of gram-negative enteric bacteria
2. It is used in the differentiation of lactose fermenting from lactose non-fermenting gram-negative bacteria
3. It is used for the isolation of coliforms and intestinal pathogens in water, dairy products and biological specimens

Preparation of MacConkey Agar

1. Suspend 49.53 grams of dehydrated medium in 1000 ml purified/distilled water
2. Heat to boiling to dissolve the medium completely
3. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes
4. Cool to 45-50°C

5. Mix well before pouring into sterile Petri plates
6. Result Interpretation on MacConkey Agar
7. **Lactose fermenting strains** grow as **red or pink** and may be surrounded by a zone of acid precipitated bile. The red colour is due to production of acid from lactose, absorption of neutral red and a subsequent colour change of the dye when the pH of medium falls below 6.8
8. **Lactose non-fermenting strains**, such as *Shigella* and *Salmonella* are **colourless** and **transparent** and typically do not alter appearance of the medium. *Yersinia enterocolitica* may appear as small, non-lactose fermenting colonies after incubation at room temperature

TABLE: 3

Colony Morphology on MacConkey Agar

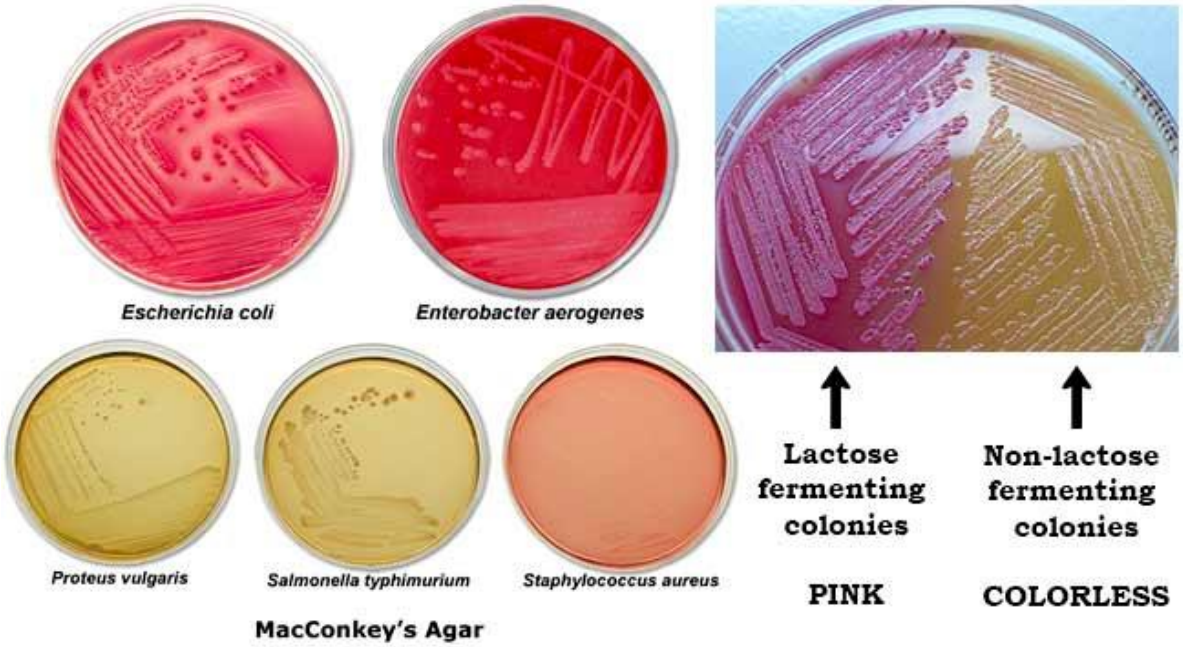
Organism	Colour	Remarks
Escherichia coli	Red/Pink	Non-mucoid
Aerobacteraerogenes	Pink	Mucoid
Enterococcus species	Red	Minute, round
Staphylococcus species	Pale pink	Opaque
Pseudomonas aeruginosa	Green-brown	Fluorescent growth

Following inoculation, the plates were incubated in a bacteriological incubator at 370° C and after overnight incubation and after 48 hours the plates were checked. If bacterial growth was obtained on the plates, the colony count was first estimated by counting the number of colonies in the streaked area of the plate and multiplying the same by 10.

A smear of an individual colony was prepared on a microscope slide, and this was stained by the gram method, dried and viewed under the oil immersion objective or a light microscope. If positive, with the presence of beta haemolysis on blood agar and growth on MacConkey agar, the growth was considered to be *staphylococcus aureus*. If coagulase test was negative, with no beta haemolysis on blood agar and no growth on MacConkey agar, the growth was considered to be a coagulase-negative staphylococcus.

Fig. 17

Colony Morphology on MacConkey Agar



Penetration (Aqueous)

The patients were instructed to use their antibiotic drops according to Regimen A or B to which they were assigned. During surgery 0.2 ml of aqueous humour was aspirated with the help of a tuberculin syringe. The anterior chamber fluid was immediately placed into a sterilized cryogenic tube and stored at **-40° C** in a refrigerator and kept frozen until analysis was performed. Each tube was marked with a patient identification number (which indicated the antibiotic that the subject was randomly assigned), the subject's initials, date of surgery, and eye (R/L).

Concentrations of topically applied fluoroquinolones were determined by use of reverse-phase high pressure liquid chromatography (HPLC) assay technique with ultraviolet–visible detector at a wavelength of 275 nm.

Chromatographic Conditions for Besifloxacin

Stationary Phase	:	Phenomenex 250×4.60 mm 5 micron
Mobile Phase	:	Acetonitrile : Water
Mobile phase ratio	:	90:10% v/v
Flow rate	:	1.0 ml/min
Sample volume	:	10µl
Detection	:	275nm using UV Visible detector
Software	:	LC Real time analysis

Result & Discussion

VIII. RESULT & DISCUSSION

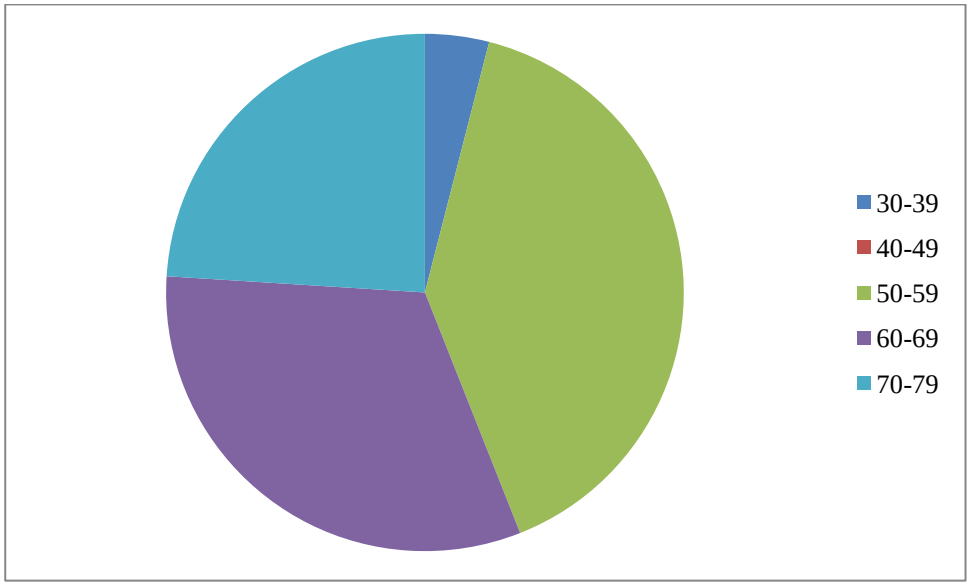
Table - 4

Categorization of patients according to Age - Regimen A

(n=25)

AGE (in yrs)	No. of PATIENTS	PERCENTAGE (%)
30-39	1	4%
40-49	0	Nil
50-59	10	40%
60-69	8	32%
70-79	6	24%
80-89	0	Nil

Fig. 18



Out of the selected 25 patients, 1 patient (4%) was in the age group of 30-39 years, no patient was in the age group of 40-49 years, 10 patients (40%) were in the age group of 50-59 years, 8 patients (32%) were in the age group of 60-69 years and 6 patients (24%) were in the age group of 70-79 years; whereas no patient was in the group of 80-89 years of age.

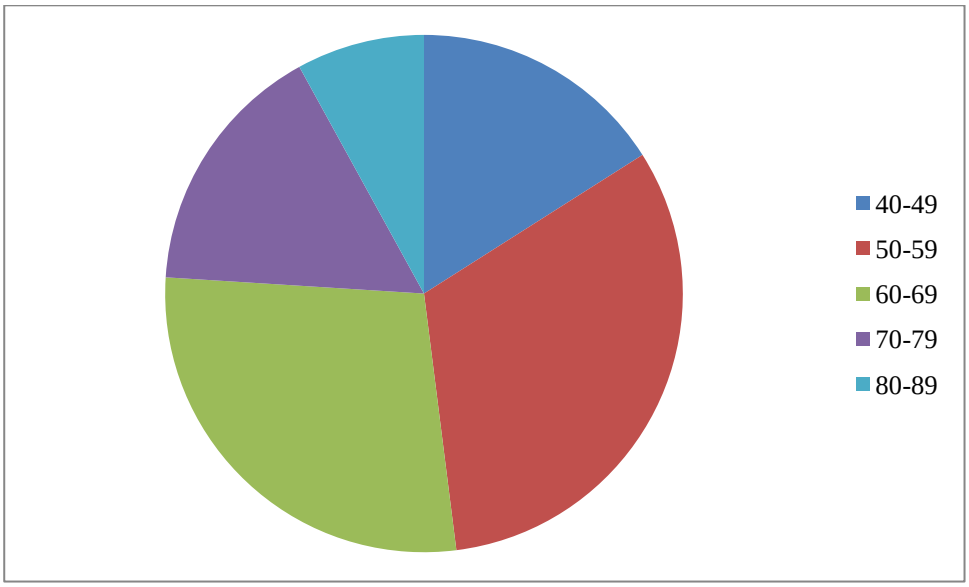
Table - 5

Categorization of patients according to Age - RegimenB

(n=25)

AGE (in yrs)	No. of PATIENTS	PERCENTAGE (%)
30-39	0	Nil
40-49	4	16%
50-59	8	32%
60-69	7	28%
70-79	4	16%
80-89	2	8%

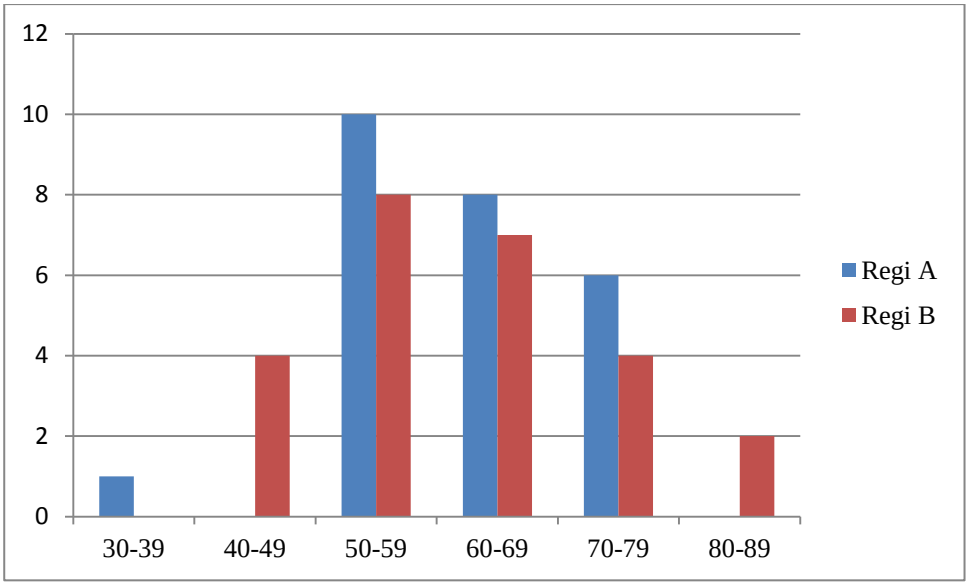
Fig. 19



Out of the selected 25 patients, no patient was in the age group of 30-39 yrs; 4 patients (16%) were in the age group of 40-49 years, 8 patients (32%) were in the age group of 50-59 years, 7 patients (28%) were in the age group of 60-69 years, 4 patients (16%) were in the age group of 70-79 years, and 2 patients (8%) were in the age group of 80-89 years.

Comparison of Age (in yrs) between Regimen A and B

Fig. 20



In Regimen – A, out of the selected 25 patients, 1 patient (4%) was in the age group of 30-39 years, no patient was in the age group of 40-49 years, 10 patients (40%) were in the age group of 50-59 years, 8 patients (32%) were in the age group of 60-69 years and 6 patients (24%) were in the age group of 70-79 years; whereas no patient was in the group of 80-89 years of age.

And in Regimen – B, out of the selected 25 patients, no patient was in the age group of 30-39 yrs; 4 patients (16%) were in the age group of 40-49 years, 8 patients (32%) were in the age group of 50-59 years, 7 patients (28%) were in the age group of 60-69 years, 4 patients (16%) were in the age group of 70-79 years, and 2 patients (8%) were in the age group of 80-89 years.

As is seen Regimen A didn't have any patient in the group of 40-49 years of age and 80-89 years, while the second Regimen didn't have any patient in the age group of 30-39 years. In the rest of the age groups both regimens had almost equal distribution of patients, thereby nullifying any possible variability.

Table- 6

Gender wise categorization of patients in Regimen – A

(n=25)

GENDER	No. of PATIENTS	PERCENTAGE (%)
Male	11	48
Female	14	56

Fig. 21

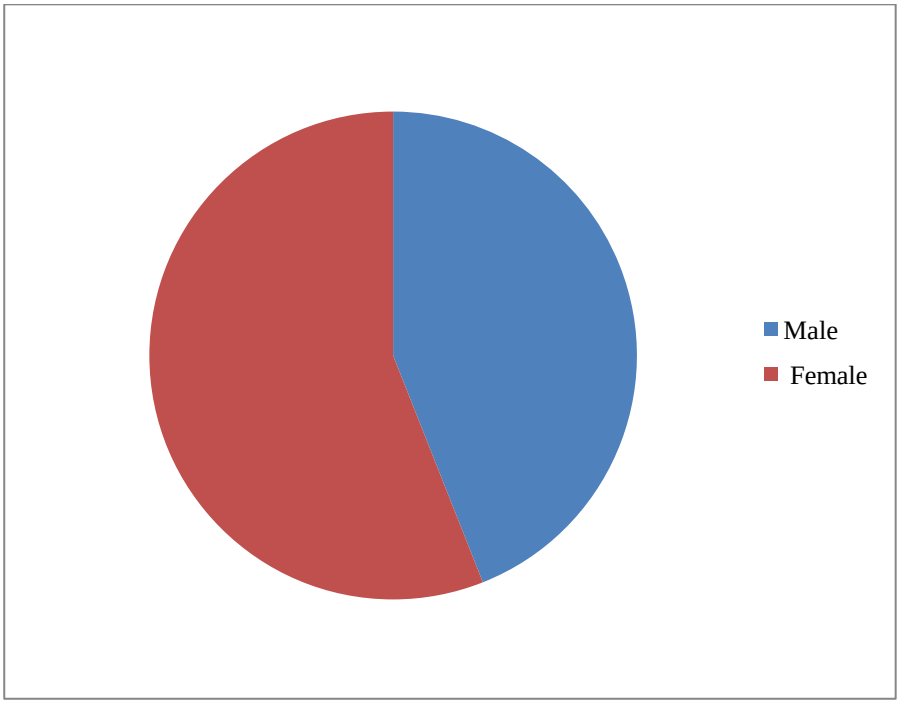


Table-7

Gender wise categorization of patients in Regimen – B(n=25)

GENDER	No.of PATIENTS	PERCENTAGE(%)
Male	13	52
Female	12	48

Fig. 22

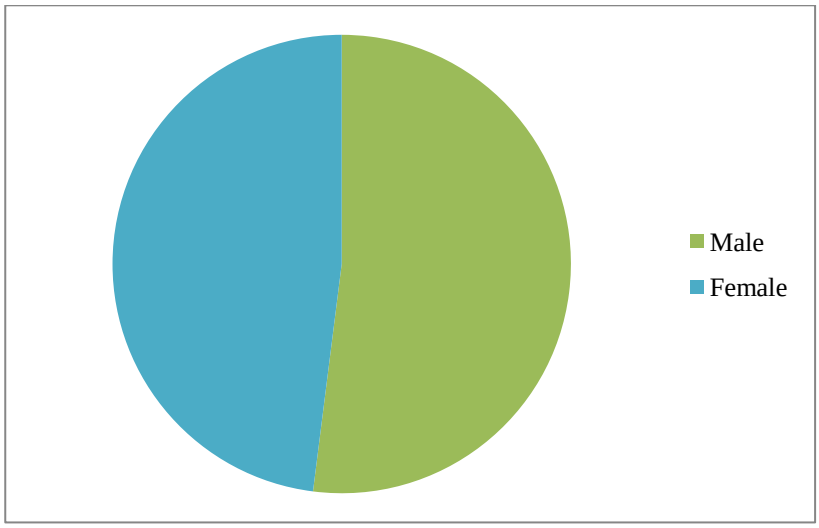
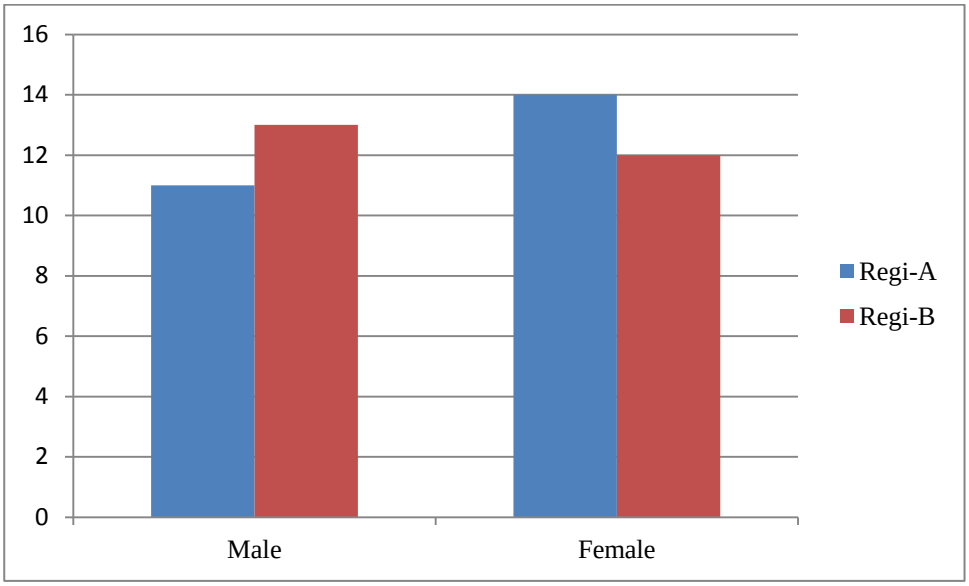


Fig. 23

Comparison between Gender classes in Regimen-A &B



In Regimen A, out of the selected 25 patients, 11 patients (48%) were males and the 14 patients (56%) were females; whereas in Regimen B, out of the selected 25 patients,13 patients (52%) were males and the 12 patients (48%) were females, showing almost equal distribution of genders in both the regimens.

Result of Associated disease conditions secondarily causing Cataract:

Cataract secondary to other disorders include not only diabetes but also hyper tension alone or hyper tension with diabetes, and anaemia. Not only do diabetics develop cataracts earlier, they are more likely to suffer complications associated with cataract surgery. For this reason, considerable research has been performed to better understand why diabetics develop lens changes.

Several concerns with diabetics: 1). Diabetics do not process glucose normally. The enzyme aldose reductase changes glucose to sorbitol through the polyol pathway. Sorbitol should be changed to fructose by the enzyme sorbitol dehydrogenase, but the sorbitol is produced faster than it can be converted to fructose, causing a buildup of sorbitol in the lens. Accumulation of sorbitol leads to increased water within the lens, changing the lens fiber array and formation of sugar cataracts.2). Osmotic stress caused by the sorbitol accumulation causes death of lens epithelial cells leading to the development of cataract.

Table- 8

Categorization of patients according to Associated diseases

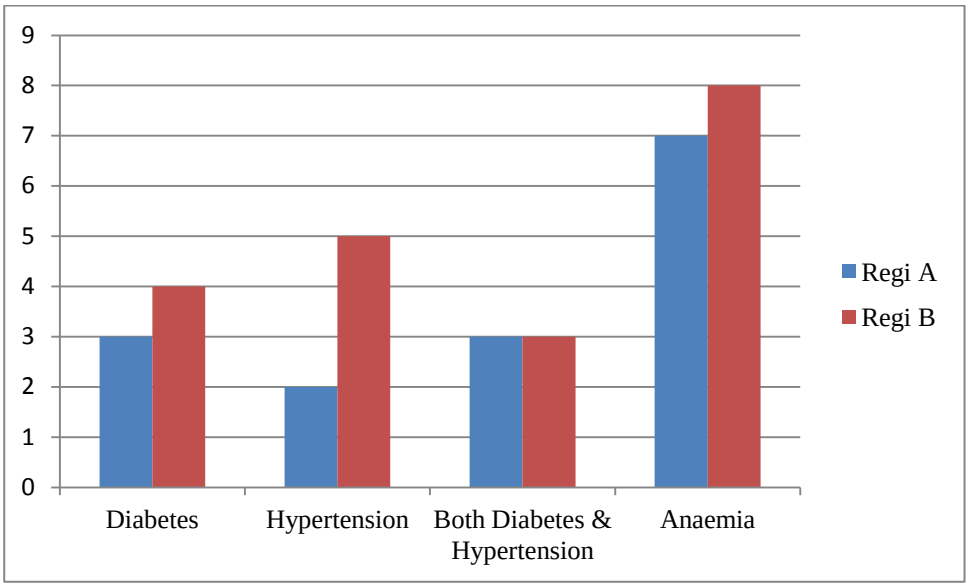
Each Regimen (n=25)

ASSOCIATED DISEASES	**REGIMEN – A**		**REGIMEN – B**	
	No. of Patients	Percentage (%)	No. of Patients	Percentage (%)
Diabetes	3	12	4	16
Hypertension	2	8	5	20
Diabetes with hypertension	3	12	3	12
Anaemia	7	28	8	32

Fig. 24

Categorization of patients according to Associated diseases

Each Regimen (n=25)



In Regimen-A, out of the selected 25 patients, 3 patients (12%) were Diabetic, and 2 patients (8%) were Hypertensive, 3 patients (12%) were both Diabetic and hypertensive and 7 patients (28%) were found to have Anemia.Out of the selected 25 patients in Regimen-B, 4 patients (16%) were Diabetic, and 5 patients (20%) were Hypertensive, 3 patients (12%) were both Diabetic and hypertensive and 8 patients (32%) were found to be Anemic.

Results of Microbial isolates study:

To study the nature and frequency of bacterial contamination during cataract surgery, we have taken preoperative smears from the conjunctiva and anterior chamber (AC) fluid aspirates during extra-capsular cataract surgery (ECCE) with posterior chamber intraocular lens (PCIOL) implantation in all the 50 eyes for aerobic and anaerobic bacteria. Any change in the bacterial strains isolated before and after cataract surgery was also studied and results presented here.

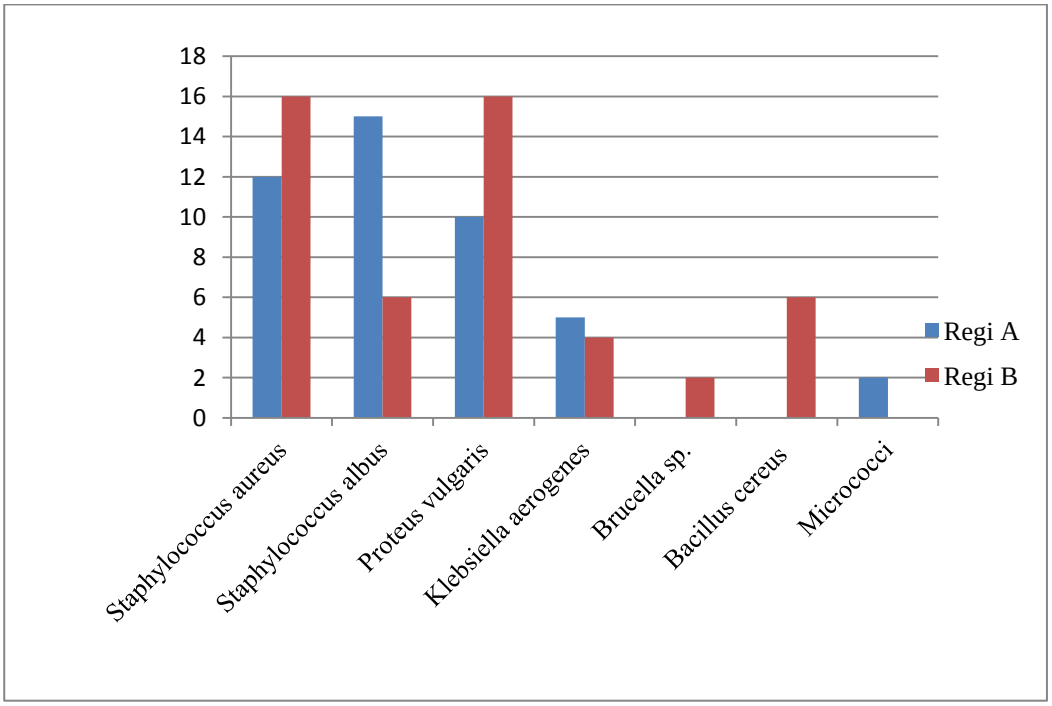
Table-9

Categorization of patients in both the study teams according to Microbes isolated

Each Regimen (n=25)

MICRO ORGANISMS ISOLATED	REGIMEN - A		REGIMEN – B	
	No. of PATIENTS	PERCENTAGE (%)	No. of PATIENTS	PERCENTAGE (%)
Staphylococcus albus	15	60 %	6	24 %
Staphylococcus aureus	12	48 %	16	64 %
Proteus vulgaris	10	40 %	16	64 %
Klebsiella aerogenes	5	20 %	4	16 %
Bacillus cereus	2	8 %	6	24 %
Brucella sps.	0	Nil	2	8 %
Micrococci	2	8 %	0	Nil

Fig. 25



In Regimen – A, out of the selected 25 patients, 15 patients (60%) had *Staphylococcus albus*, 12 patients (48%) had *Staphylococcus aureus*, 10 patients (40%) had *Proteus vulgaris*, 5 patients (20%) had *Klebsiella aerogenes* and 2 patients (8%) had *Micrococci*.

In Regimen - B, out of the selected 25 patients, 6 patients (24%) had *Staphylococcus albus*, 16 patients (64%) had *Staphylococcus aureus*, 16 patients (64%) had *Proteus vulgaris*, 4 patients (16%) had *Klebsiella aerogenes*, 6 patients (24%) had *Bacillus cereus* and 2 patients (8%) had *Brucella sps.*

Coagulase-negative *Staphylococcus* was the most common aerobe (60 %)and *S. aureus* was the most common facultative anaerobe. Of the total of 50 cases, 2 showed an organism different from other conjunctival smear. These two were *Micrococci* that was isolated under Regime – B patients. Clinically there was no endophthalmitis in any of the eyes. Factors such as preoperative antibiotic use, the antibacterial properties of aqueous, or low inoculum size could explain this. The preoperative conjunctival smear may not be useful in predicting the AC fluid contamination or outcome of cataract surgery.

It has been postulated that the anterior chamber (AC) remains sterile during cataract extraction. **Hara and coworkers (1997)**have investigated the changes in the bacterial strains present before and after cataract surgery and found that preoperative cultures are not a useful guide to predict the organism responsible for postoperative infections. But more recently other investigators have demonstrated that bacteria routinely enter the AC during cataract surgery.

Results of Types of cataracts done:

Types of Cataracts include:

- A **subcapsular cataract** occurs at the back of the lens. People with diabetes or those taking high doses of steroid medications have a greater risk of developing a subcapsular cataract – also called Posterior subcapsular cataract **(PSC)**
- A **nuclear cataract** forms deep in the central zone (nucleus) of the lens. Nuclear cataracts usually are associated with aging – also referred to as Nuclear sclerosis grade cataract **(NSG)**
- An **immature Cataract -** a cataract is considered **immature** when there are some remaining clear areas in the lens.
- A **mature Cataract** is completely opaque.

TABLE - 10

Categorization of patients according to Diagnosis in Regimen – A

(n=25)

Out of selected 25 patients, 7 patients (28%) were Posterior subcapsular cataract,10 patients (40%) were Nuclear sclerosis grade cataract and the remaining 8 patients (32%) were of Immature cataract. There was no case of Mature cataract in Regimen – A group.

DIAGNOSIS	No. of PATIENTS	PERCENTAGE (%)
Posterior subcapsular cataract (PSC)	7	28%
Nuclear sclerosis grade cataract (NSG)	10	40%
Immature cataract (IMC)	8	32%
Mature cataract (MC)	Nil	--

Fig. 26

Diagnosis-wise Categorization of Regimen- A

(n=25)

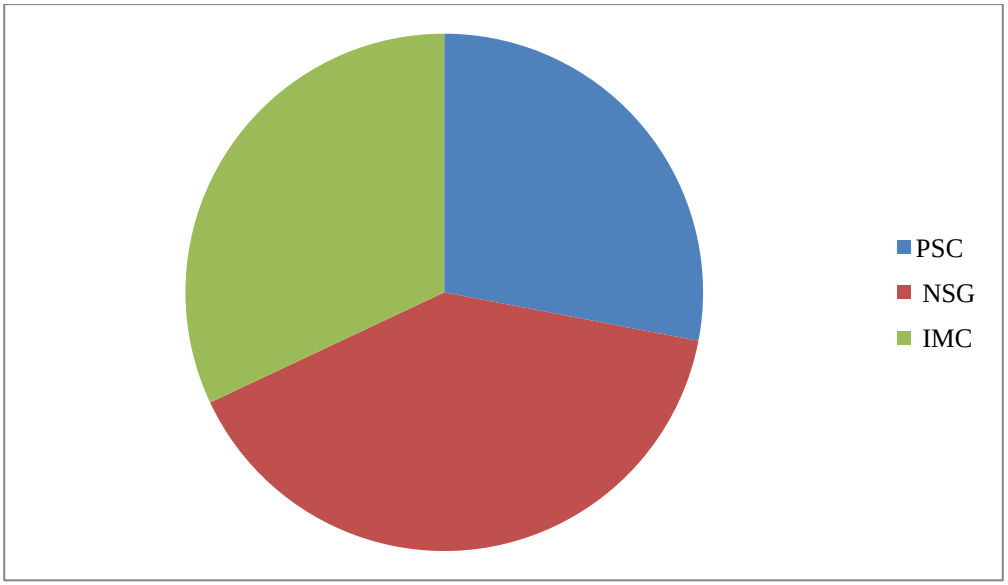


TABLE - 11

Categorization of patients according to Diagnosis in Regimen – B

(n=25)

Out of selected 25 patients, 7 patients (28%) were Posterior sub capsular cataract,13 patients (52%) were Nuclear sclerosis grade cataract, 3 patients (12%) were Immature cataract and the 2 patients (8%) were Mature cataract.

DIAGNOSIS	No OF PATIENTS	PERCENTAGE (%)
Posterior sub capsular cataract (PSC)	7	28%
Nuclear sclerosis grade cataract (NSG)	13	52%
Immature cataract (IMC)	3	12%
Mature cataract (MC)	2	8%

Fig. 27

Diagnosis-wise Categorization of Regimen- B

(n=25)

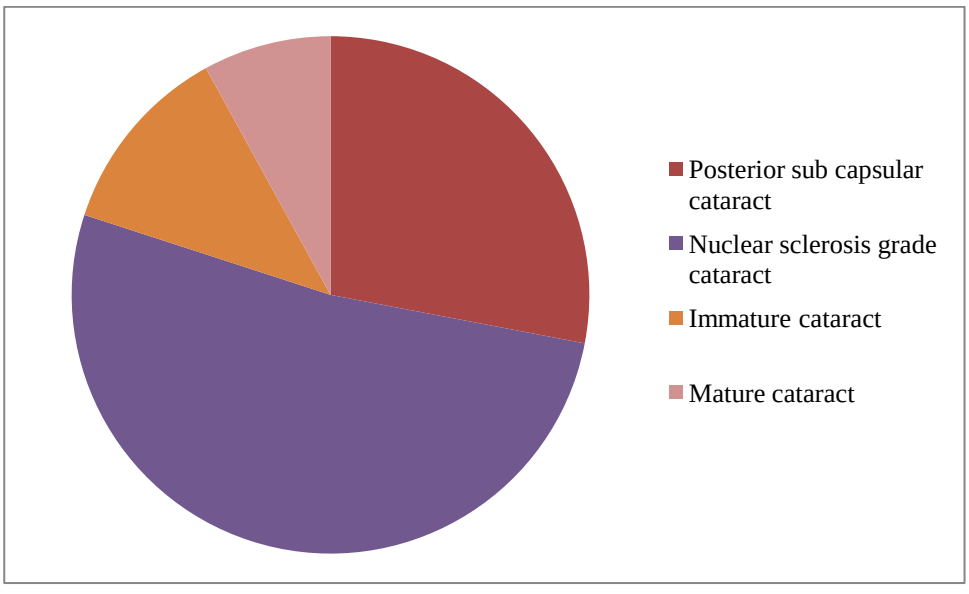
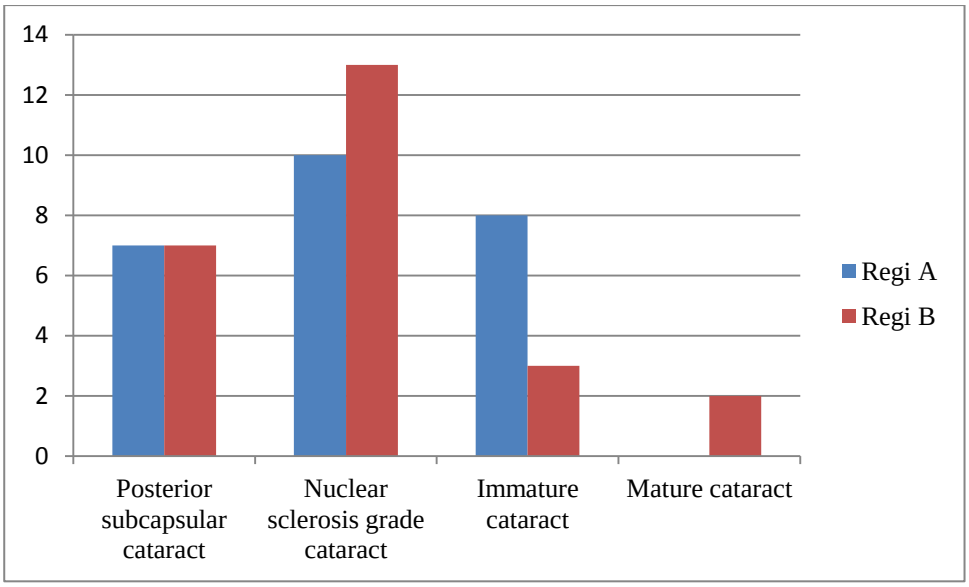


Fig. 28

Comparison between Diagnosis-wise Categories in Regimen A and Regimen B



In this study, it has been found that three types of Cataracts namely the Posterior Subcapsular Cataract (PSC), nuclear sclerosis grade cataract (NSGC) and immature senile cataract(IMSC) were the abundantly occurring types in both the study batches. Two cases of mature senile cataract (MSC) were seen in Regimen – B; whereas no case of hyper mature senile cataract (HMSC) was seen in either of the groups.

Results of the study on the penetration of Besifloxacin in the aqueous humour in anterior chamber by using a High Pressure Liquid Chromatography (HPLC) analyzer:

Table – 12

Mean Penetration of Besifloxacin - Regimen–A(n=25)

DIAGNOSIS	No. of PATIENTS	EFFICACY	PENETRATION (%)
Posterior sub capsular cataract	7	Slight microbial growth is seen in 1patient (#-24)	72.9 % (Min- 52.3%)(Max- 9.75%)
Nuclear sclerosis grade cataract	10	Nil growth	69% (Min-52.7%)(Max-93.3%)
Immature cataract	8	Slight microbial growth is seen in 1patient (Sample #-15)	69.1% (Min- 52.0%)(Max- 95%)

Table – 13

Mean Penetration of Besifloxacin in different types of Cataract - Regimen- B

(n=25)

DIAGNOSIS	No. OF PATIENTS	EFFICACY	PENETRATION (%)
Posterior sub capsular cataract	7	Slight microbial growth is seen in 2 patients (Sample #- 5 & 20)	74.8% (Min-57.4%)(Max-89.2 %)
Nuclear sclerosis grade cataract	13	Matty growth is seen in Sample # 2, 3, 14 & 16	78.4% (Min-59.1%)(Max-95.3 %)
Immature cataract	3	No Growth	70.7% (Min-31.4%)(Max- 82.3%)
Mature Cataract	2	Slight growth is seen in Sample # 6	61.9% (Min-54.7%)(Max- 86.7%)

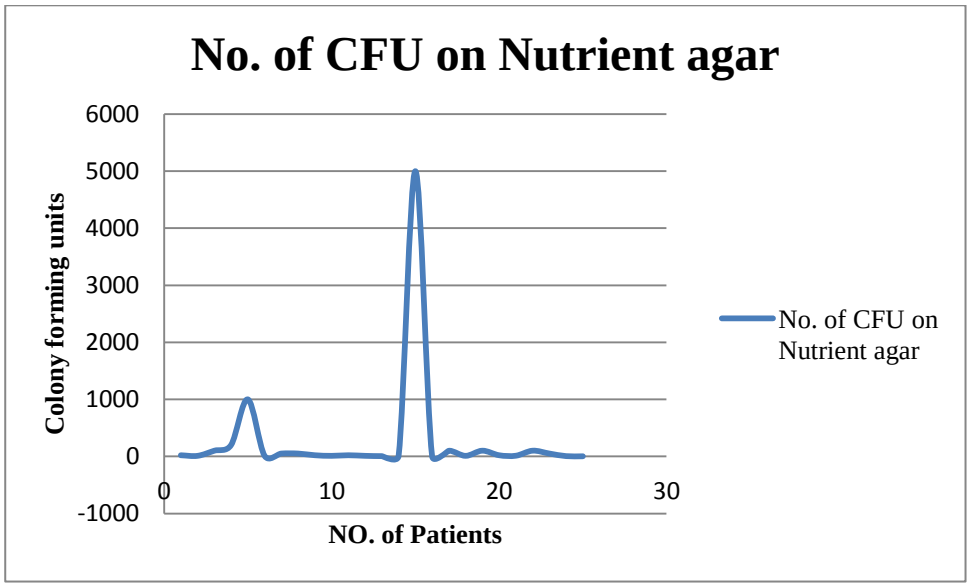
Table - 14

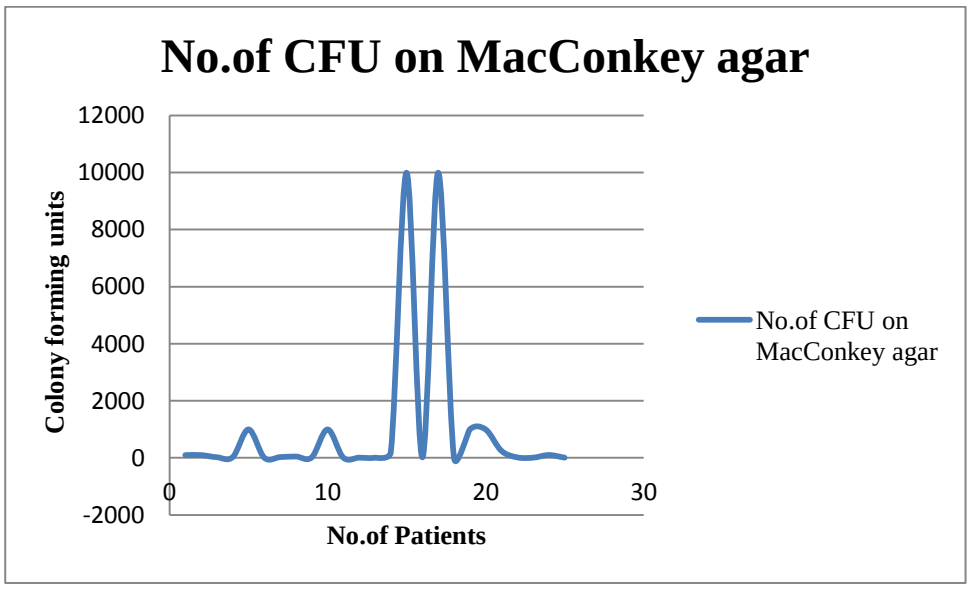
Overall Percentage of drug penetration in Aqueous humour both in Regimen-A and Regimen-B

REGIMEN–A	REGIMEN – B
70.30%	74.97%

Fig. 29

Conjunctival <u>base-line</u> bacterial load seen on Two different Agar Plates - Regimen A

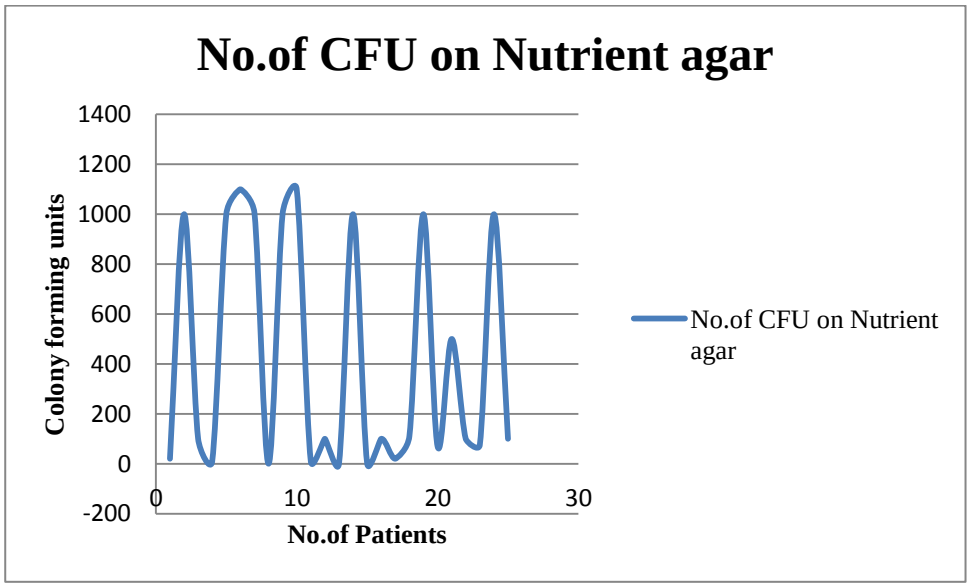


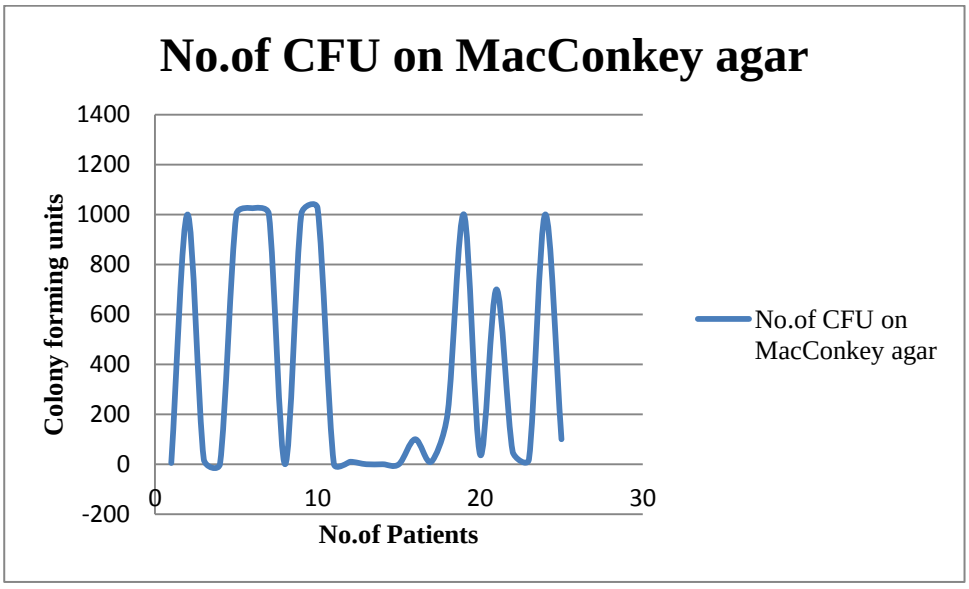


In Regimen – A, out of the 25 patients the **base line** bacterial load was found to be between 5 to 5,000 Colony Forming Units (CFUs) in Nutrient agar plate and 5 to 10,000 CFUs in MacConkey agar medium.

Fig. 30

Conjunctival <u>base-line</u> bacterial load seen on Two different Agar Plates - Regimen B

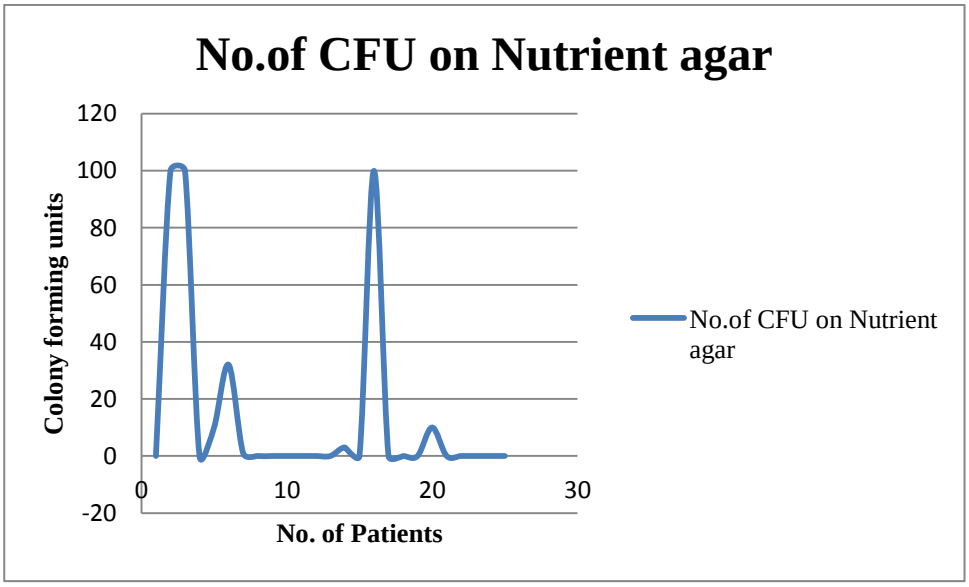




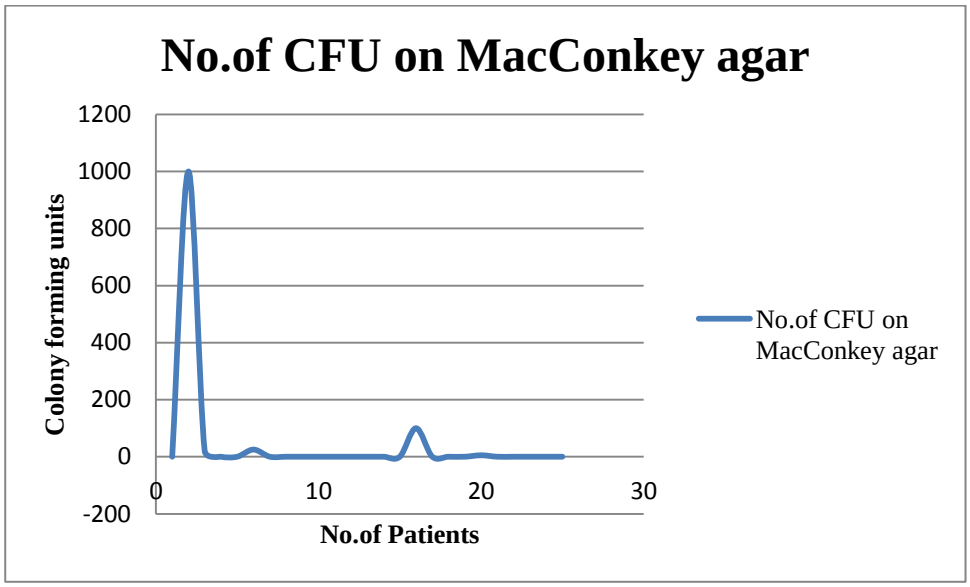
Out of the selected 25 patients, the **base line** bacterial load was found to be between 1 to 1100 Colony Forming Units in Nutrient agar and 1 to 1000 CFUs in MacConkey agar medium.

Fig. 31

Conjunctival <u>pre-operative</u> bacterial load seen on two different Agar Plates after Besifloxacin administration – Regimen B



Out of the selected 25 patients, the **pre-operative** bacterial load was found to be between 1 to 100 CFUs in Nutrient agar and 1 to 1000 Colony Forming Units in MacConkey medium.



In the Colony Forming Units (CFUs) count on Nutrient agar plates and MacConkey agar plates, the base-line count in patients of Regimen-A was found to be between5 to 5,000 CFUs in Nutrient agar and 5 to 10,000 CFUs in MacConkey agar medium. In the case of Regimen-B, the base line bacterial load was found to be between 1 to 1100 CFUs in Nutrient agar and 1 to 1000 CFUs in MacConkey agar medium.

Whereas the pre-operative colony count showed ‘ nil growth’ in Regimen-A and a drastic reduction in case of pulse mode of administration *i.e.*, in Regimen-B was noticed. Thereby it is seen that Regimen A shows a better antimicrobial effect locally on the cornea whereas the aqueous humor penetration was better in the case of Regimen B. END

Conclusion

IX. CONCLUSION

Prophylactic antibiotic eye drops are routinely used to prevent postoperative complications like endophthalmitis. Fluoroquinolones in particular have been used extensively due to their excellent activity against ocular pathogens, including both Gram-positive and Gram-negative bacteria. Selection of the most appropriate antibiotic should be based on considerations of pharmacodynamic and pharmacokinetic properties; namely, an agent with a broader spectrum, lower minimum inhibitory concentration (MIC) against pathogens, and better achievable concentration levels in the intraocular tissues should be the agent of choice to achieve optimal efficacy.

To summarize current understanding of antibiotic prophylaxis in cataract surgery, with particular emphasis on available evidence and change in practice patterns over the past decade, the fourth generation fluoroquinolone - Besifloxacin was taken up here for determining its antimicrobial potency in the cornea and its ability to penetrate into the anterior chamber.

- Results of the present study revealed no association between the grade of cataract and risk factors such as age, duration of diabetes or gender as evident from the correlation coefficient analysis.

- NSGC - the Nuclear sclerosis graded cataract emerged as the most commonly found cataract in this hospital. This is followed by PSC – the Posterior subcapsular cataract.

- Besifloxacin, the 4^{th} Gen fluoroquinolone, penetrates the conjunctiva when applied topically to the extent of 70.30 % in conventional mode of administration and 74.97 % in the pulse mode of instillation.

- With the normal antimicrobial range of Besifloxacin, it is expected to prevent post-surgical endophthalmitis to a great extent.

- It’s clearly seen that pulse mode of administration of topical antibiotics an hour preceding surgery shows better penetration of the drug than administered with conventional style of two days prior to surgery.

However, there are few studies that have clearly assessed the superiority of one drug over the other among the newer fourth-generation fluoroquinolones, including moxifloxacin and gatifloxacin, when used in humans. Based on current evidence from this study, the recommended measures for endophthalmitis prophylaxis are preoperative topical instillation of fluoroquinolone antibiotic and if need be, intra caramel injection at the end of cataract surgery.

WHETHER CATARACTS BE PREVENTED?

Protecting the eyes from the sun's radiation with UV-filtering sunglasses may help slow the progression of cataracts. Controlling other eye diseases and quitting smoking if it is a smoker will also decrease the risk.

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Appendices

XI. APPENDICES

PROFORMA-1

INFORMED CONSENT FORM

PATIENT NAME : DATE:

AGE/ SEX : REF NO:

I was explained about the description of the research study and they have answered all the questions I have at this time.

I freely volunteer to participate in this study. I understand that I do not have to take part in this study and that my refusal to participate will involve no penalty. Further I understand that I am free to discontinue participation from this study at any time.

Clinician's Name with signature Signature of the Patient

அறிவிக்கப்பட்ட முடிவு

நோயாளியின் பெயர் : தேதி :

வயது/ பாலினம் : பதிவு எண் :

எனக்கு ஆராய்ச்சி ஆய்வு விளக்கம் பற்றி முழுவதுமாக விளக்கியதோடு, நான் கேட்ட அனைத்து சந்தேகங்களுக்கும் திருப்திகரமாக பதில் அளித்தனர்.

நான் முழுமனதாக இந்த ஆய்வில் பங்கேற்க சம்மதிக்கிறேன். இந்த ஆய்வில் என் பங்கேற்பு கட்டாயபடுத்தபடவில்லை எனவும், இதில் நான் பங்கேற்க்க மறுப்பதினால் எனக்கு அபராதம் எதுவும் இல்லை எனவும், மற்றம் இந்த ஆய்வில் இருந்து எந்த நேரமும் நான் விலகிக்கொள்ள எனக்கு உரிமை உண்டு என்பதையும், நான் புரிந்து கொண்டேன்.

மருத்துவரின் பெயர் **நோயாளியின் கையொப்பம்**

PROFORMA-2

PATIENT PARTICULARS

NAME :

AGE :

SEX :

DATE :

REFERENCE NO :

CONSULTANT'S NAME :

MEDICAL HISTORY :

PRESENT COMPLAINTS :

ON EXAMINATION :

DIAGNOSIS :

OTHER INVESTIGATIONS :

PROFORMA-3

SURGICAL PROCEDURE PARTICULARS

TYPE OF OPERATION :

ANESTHESIA : Yes / No / Standby

DATE OF SURGERY :

EYE : Right / Left

TYPE OF LENS : POWER :

DRUG : Besifloxacin E/D

DOSAGE REGIMEN : A / B

COURSE IN HOSPITAL :

PROFORMA-4

MEDICATION DETAILS

REGIME-A

S,NO	DATE	MEDICATION	QTY/DOSE	TIMING	ROUTE	FREQUENCY
1		Besifloxacin				
2						

REGIME-B

S.NO	DATE	MEDICATION	QTY/DOSE	TIMING	ROUTE	FREQUENCY
1		Besifloxacin				
2						
3						
4						
5						
6						